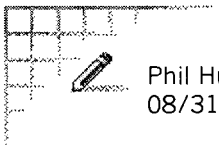
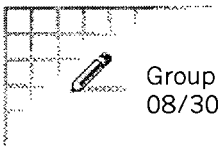
EPA Pesticide Petition No. 8F5032



Phil Hutton
08/31/2000 11:12 PM

To: susanne cerrelli/dc/usepa/us, Anne Ball/DC/USEPA/US
cc:
Subject: Pesticide Product Registrations; Conditional Approval

---------- Forwarded by Phil Hutton/DC/USEPA/US on 08/31/2000 11:12 PM ----------



Group Envsubset
08/30/2000 03:42 PM

Please respond to epa-pest2@valley.rtpnc.epa.gov

To: epa-pest2@valley.rtpnc.epa.gov
cc:
Subject: Pesticide Product Registrations; Conditional Approval

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[Federal Register: August 30, 2000 (Volume 65, Number 169)]
[Notices]
[Page 52732-52733]
>From the Federal Register Online via GPO Access [wais.access.gpo.gov]
[DOCID:fr30au00-76]

ENVIRONMENTAL PROTECTION AGENCY

[OPP-30480A; FRL-6740-3]

Pesticide Product Registrations; Conditional Approval

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: This notice announces Agency approval of applications submitted by AgraQuest, Inc., to conditionally register the pesticide products Serenade^{TM} Biofungicide Wettable Powder and QST 713 Technical containing a new active ingredient not included in any previously registered products pursuant to the provisions of section

3(c)(7)(C) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended.

FOR FURTHER INFORMATION CONTACT: By mail: Susanne Cerrelli, Biopesticides and Pollution Prevention Division (7511C), Office of Pesticide Programs, Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460; telephone number: 703-308-8077; e-mail address: cerrelli.susanne@epa.gov.

SUPPLEMENTARY INFORMATION:

I. General Information

A. Does this Action Apply to Me?

You may be affected by this action if you are an agricultural producer, food manufacturer, or pesticide manufacturer. Potentially affected categories and entities may include, but are not limited to:

Categories	NAICS codes	Examples of potentially affected entities
Industry	111	Crop production
	112	Animal production
	311	Food manufacturing
	32532	Pesticide manufacturing

This listing is not intended to be exhaustive, but rather provides a guide for readers regarding entities likely to be affected by this action. Other types of entities not listed in the table could also be affected. The North American Industrial Classification System (NAICS) codes have been provided to assist you and others in determining whether or not this action might apply to certain entities. If you have questions regarding the applicability of this action to a particular entity, consult the person listed under FOR FURTHER INFORMATION CONTACT.

B. How Can I Get Additional Information, Including Copies of this Document and Other Related Documents?

1. Electronically. You may obtain electronic copies of this document, and certain other related documents that might be available electronically, from the EPA Internet Home Page at http://www.epa.gov/. To access this

[[Page 52733]]

document, on the Home Page select ``Laws and Regulations,'' ``Regulations and Proposed Rules,'' and then look up the entry for this document under the ``Federal Register--Environmental Documents.'' You

can also go directly to the Federal Register listings at http://www.epa.gov/fedrgstr/.

To access a fact sheet which provides more detail on this registration, go to the Home Page for the Office of Pesticide Programs at http://www.epa.gov/pesticides/, and select "fact sheet."

2. In person. The Agency has established an official record for this action under docket control number OPP-30480A. The official record consists of the documents specifically referenced in this action, any public comments received during an applicable comment period, and other information related to this action, including any information claimed as Confidential Business Information (CBI). This official record includes the documents that are physically located in the docket, as well as the documents that are referenced in those documents. The public version of the official record does not include any information claimed as CBI. The public version of the official record, which includes printed, paper versions of any electronic comments submitted during an applicable comment period, is available for inspection in the Public Information and Records Integrity Branch (PIRIB), Rm. 119, Crystal Mall #2, 1921 Jefferson Davis Hwy., Arlington, VA, from 8:30 a.m. to 4 p.m., Monday through Friday, excluding legal holidays. The PIRIB telephone number is (703) 305-5805.

In accordance with section 3(c)(2) of FIFRA, a copy of the approved label, the list of data references, the data and other scientific information used to support registration, except for material specifically protected by section 10 of FIFRA, are available for public inspection in the Public Information and Records Integrity Branch, Information Resources and Services Division (7502C), Office of Pesticide Programs, Environmental Protection Agency, Rm. 119, Crystal Mall #2, Arlington, VA (703) 305-5805). Requests for data must be made in accordance with the provisions of the Freedom of Information Act and must be addressed to the Freedom of Information Office (A-101), 1200 Pennsylvania Ave., NW., Washington, DC 20460. Such requests should: Identify the product name and registration number and specify the data or information desired.

A paper copy of the fact sheet, which provides more detail on this registration, may be obtained from the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161.

II. Did EPA Conditionally Approve the Application?

A conditional registration may be granted under section 3(c)(7)(C) of FIFRA for a new active ingredient where certain data are lacking, on condition that such data are received by the end of the conditional registration period and do not meet or exceed the risk criteria set forth in 40 CFR 154.7; that use of the pesticide during the conditional registration period will not cause unreasonable adverse effects; and that use of the pesticide is in the public interest. The Agency has considered the available data on the risks associated with the proposed use of Bacillus subtilis strain QST 713, and information on social, economic, and environmental benefits to be derived from such use. Specifically, the Agency has considered the nature and its pattern of use, application methods and rates, and level and extent of potential exposure. Based on these reviews, the Agency was able to make basic health and safety determinations which show that use of Bacillus

subtilis strain QST 713 during the period of conditional registration will not cause any unreasonable adverse effect on the environment, and that use of the pesticide is, in the public interest.

Consistent with section 3(c)(7)(C) of FIFRA, the Agency has determined that these conditional registrations are in the public interest. Use of the pesticides are of significance to the user community, and appropriate labeling, use directions, and other measures have been taken to ensure that use of the pesticides will not result in unreasonable adverse effects to man and the environment.

III. Approved Applications

1. Applications approved and published. EPA published a notice in the Federal Register of June 16, 1999 (64 FR 32231) (FRL-6084-5), announcing that AgraQuest, Inc., 1105 Kennedy Place, Davis, CA 95616), (now located at 1530 Drew Ave., Davis, CA), had submitted an application to conditionally register the pesticide product, QST 713 Technical, microbial fungicide (EPA File Symbol 69592-L), containing the QST 713 strain of dried Bacillus subtilis at 5%. Presently, the QST 713 Technical, microbial fungicide contains the QST 713 strain of Bacillus subtilis at 14.6%, an active ingredient not included in any previously registered product.

2. Application approved but not published. AgraQuest, Inc., submitted an application to EPA to register the pesticide product Serenade^{TM} Biofungicide Wettable Powder (EPA File Symbol 69592-U) containing the same chemical at 14.6%. However, since the notice of receipt of the application to register the product as required by section 3(c)(4) of FIFRA, as amended, did not publish in the Federal Register, interested parties may submit comments on or before September 29, 2000 for this product only.

The applications were conditionally approved on June 20, 2000 for an end-use product and a technical listed below:

1. Serenade^{TM} Biofungicide Wettable Powder, for use on cherries, cucurbits, grapes, hops, leafy vegetables (except Brassica), peanuts, pepper, potato, tomato, and walnuts (EPA Registration Number 69592-4)

2. QST 713 Technical, for use in manufacturing and formulating end-use products to control various fungal plant pathogens and terrestrial use (EPA Registration Number 69592-5).

List of Subjects

Environmental protection, Pesticides and pests.

Dated: August 17, 2000.
Kathleen D. Knox,
Acting Director, Biopesticides and Pollution Prevention Division,
Office of Pesticide Programs.
[FR Doc. 00-21921 Filed 8-29-00; 8:45 am]
BILLING CODE 6560-50-F

AgraQuest, Inc.

1530 Drew Avenue
Davis, CA 95616-6320
tel. 530.750.0150
fax. 530.750.0153
agraquest@agraquest.com
www.agraquest.com

Innovative natural product solutions for pest management

July 14, 2000

Ms. Susanne Cerrelli
Biopesticides Pollution Prevention Division
Office of Pesticide Programs (7511C)
U. S. Environmental Protection Agency
Room 912, Crystal Mall 2
1921 Jefferson Davis Highway
Arlington, VA 22202

RE: Authorized Agents for AgraQuest, Inc.

Dear Ms. Cerrelli:

This letter is written to clarify the status of authorized agents for AgraQuest, per your request in your e-mail message of July 10, 2000. As I mentioned in my July 7 e-mail to you, I am requesting that all correspondence from the Agency be sent directly to me at the AgraQuest address shown above, regardless of the product.

In terms of the respective agents with whom we have a business relationship, Dr. E. M. Bellet of CCII in Overland Park, KS, remains our agent for Serenade Biofungicide Wettable Powder, EPA Reg. No. 69592-4. Dr. Bellet will remain involved with discussions regarding this product, but once all outstanding issues have been resolved, he will no longer represent AgraQuest.

Ms. Amy Roberts of TSG in Washington D.C. is our agent for all other products e.g., Serenade Organic and QST 2808 Technical and future AgraQuest products. In her role as agent to AgraQuest she will provide guidance to AgraQuest on registration issues, however, I, or other AgraQuest employees as appropriate will be the principle contact to the Agency for AgraQuest business. For this reason I am requesting that all correspondence be sent directly to me. There may be occasions that I will ask Amy Roberts to come by your office and pick up documents so that she can send them to us via express mail, but I will let you know in advance when this may happen. In any event, I will ensure that the proper agent has a copy of all relevant regulatory correspondence.

I hope this clarifies the situation for you relative to our agents and the products they represent for AgraQuest. If you have questions, please don't hesitate to contact me at the number shown above, extension 29.

Sincerely,

Jennifer Ryder Fox

Jennifer Ryder Fox, PhD
Director, Regulatory Affairs

cc: Dr. Pam Marrone, President/CEO, AgraQuest, Davis, CA

RECEIVED
8/3/2000

Michele Knorr/DC/USEPA/US@EPA on 03/21/200[illegible] 2:16:54 PM

To: Susanne Cerrelli/DC/USEPA/US@EPA
cc:
Subject: Re: tolerance ex. for Qst 713 strain of B. subtilus -Reply -Reply

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I concur on the revisions. Thank you for incorporating my comments.

Michele

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 180

[OPP–300997; FRL–6555–3]

RIN 2070–AB78

Bacillus subtilus Strain QST 713; Exemption from the Requirement of a Tolerance

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: This regulation establishes an exemption from the requirement of a tolerance for residues of the *Bacillus subtilus* strain QST 713 in or on all raw agricultural commodities when applied/used according to label instructions. AgraQuest, Inc. submitted a petition to EPA under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended by the Food Quality Protection Act of 1996 (FQPA), requesting an exemption from the requirement of a tolerance. This regulation eliminates the need to establish a maximum permissible level for residues of *Bacillus subtilus* strain QST 713.

DATES: This regulation is effective [*insert date of publication in the* **Federal Register**]. Objections and requests for hearings, identified by docket control number [OPP–300997], must be received by EPA, on or before [*insert date 60 days after date of publication in the* **Federal Register**].

ADDRESSES: Written objections and hearing requests may be submitted by mail, electronically, or in person. Please follow the detailed instructions for each method as provided in Unit IX. of the "SUPPLEMENTARY INFORMATION." To ensure proper receipt by EPA, your objections and hearing requests must identify docket control number [OPP–300997] in the subject line on the first page of your response.

FOR FURTHER INFORMATION CONTACT: By mail: Susanne Cerrelli, c/o Product Manager (PM) 90, Biopesticides and Pollution Prevention Division (7511C), Environmental Protection Agency, Ariel Rios Bldg., 1200 Pennsylvania Ave., NW., Washington, DC 20460; telephone number: (703) 308–8077; and e-mail address: cerrelli.susanne@epa.gov.

SUPPLEMENTARY INFORMATION:

OOP-0574

I. General Information

A. Does this Action Apply to Me?

You may be affected by this action if you are an agricultural producer, food manufacturer, or pesticide manufacturer. Potentially affected categories and entities may include, but are not limited to:

Categories	NAICS codes	Examples of potentially affected entities
Industry	111 112 311 32532	Crop production Animal production Food manufacturing Pesticide manufacturing

This listing is not intended to be exhaustive, but rather provides a guide for readers regarding entities likely to be affected by this action. Other types of entities not listed in the table could also be affected. The North American Industrial Classification System (NAICS) codes have been provided to assist you and others in determining whether or not this action might apply to certain entities. If you have questions regarding the applicability of this action to a particular entity, consult the person listed under "FOR FURTHER INFORMATION CONTACT."

B. How Can I Get Additional Information, Including Copies of this Document and Other Related Documents?

1. *Electronically.* You may obtain electronic copies of this document, and certain other related documents that might be available electronically, from the EPA Internet Home Page at http://www.epa.gov/. To access this document, on the Home Page select "Laws and Regulations" and then look up the entry for this document under the "**Federal Register**--Environmental Documents." You can also go directly to the **Federal Register** listings at http://www.epa.gov/fedrgstr/.

2. *In person.* The Agency has established an official record for this action under docket control number OPP–300997. The official record consists of the documents specifically referenced in this action, and other information related to this action, including any information claimed as Confidential Business Information (CBI). This official record includes the documents that are physically located in the docket, as well as the documents that are referenced in those documents. The public version of the official record does not include any information claimed as CBI. The public version of the official record, which includes printed, paper versions of any electronic comments submitted during an applicable comment period is available for inspection in the Public Information and Records Integrity Branch (PIRIB), Rm. 119, Crystal Mall #2 (CM #2), 1921 Jefferson Davis Hwy., Arlington, VA, from 8:30 a.m. to 4 p.m., Monday through Friday, excluding legal holidays. The PIRIB telephone number is (703) 305–5805.

II. Background and Statutory Findings

In the **Federal Register** of April 26, 1999 (64 FR 20295) (FRL–6074–8), EPA issued a notice pursuant to section 408 of FFDCA, 21 U.S.C. 346a(e), as amended by FQPA (Public Law 104–170) announcing the filing of a pesticide tolerance petition by AgraQuest, Inc., 1530 Drew Ave., Davis California 95616. This notice included a summary of the petition prepared by the petitioner AgraQuest, Inc. There were no comments received in response to the notice of filing.

The petition requested that 40 CFR part 180 be amended by establishing an exemption from the requirement of a tolerance for residues of *Bacillus subtilus* strain QST 713.

III. Risk Assessment

New section 408(c)(2)(A)(i) of FFDCA allows EPA to establish an exemption from the requirement for a tolerance (the legal limit for a pesticide chemical residue in or on a food) only if EPA determines that the tolerance is "safe." Section 408(c)(2)(A)(ii) defines "safe" to mean that "there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information." This includes exposure through drinking water and in residential settings, but does not include occupational exposure. Section 408(b)(2)(C) requires EPA to give special consideration to exposure of infants and children to the pesticide chemical residue in establishing a tolerance and to "ensure that there is a reasonable certainty that no harm will result to infants and children from aggregate exposure to the pesticide chemical residue...." Additionally, section 408(b)(2)(D) requires that the Agency consider "available information" concerning the cumulative effects of a particular pesticide's residues and "other substances that have a common mechanism of toxicity."

EPA performs a number of analyses to determine the risks from aggregate exposure to pesticide residues. First, EPA determines the toxicity of pesticides. Second, EPA examines exposure to the pesticide through food, drinking water, and through other exposures that occur as a result of pesticide use in residential settings.

IV. Toxicological Profile

Consistent with section 408(b)(2)(D) of FFDCA, EPA has reviewed the available scientific data and other relevant information in support of this action and considered its validity, completeness, and reliability and the relationship of this information to human risk. EPA has also considered available information concerning the variability of the sensitivities of major identifiable subgroups of consumers, including infants and children.

A battery of tests determined that QST 713 Technical product is not pathogenic and has no significant toxicity. The acute oral toxicity/

pathogenicity, acute pulmonary toxicity/pathogenicity and acute intravenous toxicity/pathogenicity studies demonstrated no significant toxicity and a lack of pathogenicity. The dermal toxicity and eye irritation studies resulted in a Toxicity Category III classification. The acute dermal irritation study resulted in a Toxicity Category IV classification. *Bacillus subtilis* strain QST 713 is a ubiquitous organism in the environment and there have been no reports of the organism affecting the immune system. The submitted toxicity/pathogenicity studies in rodents with *Bacillus subtilis* strain QST 713 indicated that following several routes of exposure, the immune system is still intact and able to process and clear the active ingredient. As would be expected for any microbial pesticide, QST 713 did elicit a very mild delayed hypersensitivity response and is considered a potential dermal sensitizer. Further, although it is not known whether strain QST 713 does, the species is known to produce the enzyme subtilisin which has been reported to produce allergenic or hypersensitivity reactions to individuals repeatedly exposed to the enzyme in industrial settings. The use of personal protective equipment required for applicators and other handlers mitigates the hypersensitivity risk by minimizing exposure. No hypersensitivity risk is expected for dietary exposure due to the low likelihood that any significant residues will occur on treated food.

V. Aggregate Exposures

In examining aggregate exposure, FFDCA section 408 directs EPA to consider available information concerning exposures from the pesticide residue in food and all other non-occupational exposures, including drinking water from ground water or surface water and exposure through pesticide use in gardens, lawns, or buildings (residential and other indoor uses).

A. Dietary Exposure

Dietary exposure to the microbial pesticide is likely to occur. The lack of acute oral toxicity/pathogenicity, and the ubiquitous nature of the microbial, support the establishment of an exemption from the requirement of a tolerance for *Bacillus subtilus* strain QST 713.

1. *Food.* Dietary exposure to the microbial is expected to be minimal. In additon, standard practices of washing, peeling, cooking, or processing fruits and vegetables will reduce residues of *Bacillus subtilis* strain QST 713 and further minimize dietary exposure. The risk posed to adults, infants, and children is likely to be minimal, because of the low acute oral toxicity/pathogenicity potential of the microbial pesticide.

2. *Drinking water exposure.* Oral exposure, at very low levels, may occur from ingestion of drinking water. Drinking water is not being screened for *Bacillus subtilis* strain QST 713 as a potential indicator of microbial contamination. Both percolation through soil and municipal treatment of drinking water would reduce the possibility of

exposure to the bacterial active ingredient through drinking water. If oral exposure should occur through drinking water, the Agency concludes that such exposure would present minimal risk due to the lack of acute oral toxicity/pathogenicity and the ubiquitous nature of the microbe.

B. Other Non-Occupational Exposure

The use sites proposed are for agricultural sites. Dermal and inhalation exposure is expected to be limited to those who apply or handle the pesticide in orchards and farms. *Bacillus subtilis* presence is ubiquitous in the environment and the use of this product is not expected to increase dermal or inhalation exposure in non-occupational settings.

VI. Cumulative Effects

No mechanism of toxicity in mammals has been identified for *Bacillus subtilis* strain QST 713. Therefore no cumulative effect with other related organisms is anticipated. Because the data available demonstrate a low toxicity/pathogenicity potential of the active ingredient, the likelihood of adverse dietary effects is expected to be minimal.

VII. Determination of Safety for U.S. Population, Infants and Children

Based on the acute toxicity information discussed above, EPA concludes that there is a reasonable certainty that no harm will result from aggregate exposure to the United States population, including infants and children, to residues of *Bacillus subtilis* strain QST 713. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. The Agency has arrived at this conclusion because, the data available on *Bacillus subtilis* strain QST 713 demonstrate a low toxicity/pathogenicity potential. *Bacillus subtilis* is not a human pathogen and has not been implicated in human disease, but has been isolated as a rare contaminant from human infections. Risk of increased exposure is likely only to exist for pesticide applicators and manufacturers of the product. The Agency has imposed appropriate risk mitigation measures to protect the workers via the use of protective clothing.

VIII. Other Considerations

A. Endocrine Disruptors

The Agency has no information to suggest that *Bacillus subtilis* strain QST 713 has an effect on the immune and endocrine systems. No specific tests have been conducted with *Bacillus subtilis* strain QST 713 to determine such effects. However, the submitted toxicity/pathogenicity studies in rodents indicated that following several routes of exposure, the immune system is still intact and able to process and clear the active ingredient. *Bacillus subtilis* strain QST 713 is a ubiquitous organism in the environment and there have been no reports

of the organism affecting endocrine system. Therefore, it is unlikely that this organism would have estrogenic or endocrine effects because it is practically non-toxic to mammals.

B. Analytical Method(s)

The Agency proposes to establish an exemption from the requirement of a tolerance without any numerical limitation; therefore the Agency has concluded that an analytical method is not required for enforcement purposes for *Bacillus subtilis* strain QST 713.

C. Codex Maximum Residue Level

There are no CODEX values for *Bacillus subtilis* strain QST 713.

IX. Objections and Hearing Requests

Under section 408(g) of the FFDCA, as amended by the FQPA, any person may file an objection to any aspect of this regulation and may also request a hearing on those objections. The EPA procedural regulations which govern the submission of objections and requests for hearings appear in 40 CFR part 178. Although the procedures in those regulations require some modification to reflect the amendments made to the FFDCA by the FQPA of 1996, EPA will continue to use those procedures, with appropriate adjustments, until the necessary modifications can be made. The new section 408(g) provides essentially the same process for persons to "object" to a regulation for an exemption from the requirement of a tolerance issued by EPA under new section 408(d), as was provided in the old FFDCA sections 408 and 409. However, the period for filing objections is now 60 days, rather than 30 days.

A. What Do I Need to Do to File an Objection or Request a Hearing?

You must file your objection or request a hearing on this regulation in accordance with the instructions provided in this unit and in 40 CFR part 178. To ensure proper receipt by EPA, you must identify docket control number OPP–300997 in the subject line on the first page of your submission. All requests must be in writing, and must be mailed or delivered to the Hearing Clerk on or before [*insert date 60 days after date of publication in the* **Federal Register**].

1. *Filing the request.* Your objection must specify the specific provisions in the regulation that you object to, and the grounds for the objections (40 CFR 178.25). If a hearing is requested, the objections must include a statement of the factual issues(s) on which a hearing is requested, the requestor's contentions on such issues, and a summary of any evidence relied upon by the objector (40 CFR 178.27). Information submitted in connection with an objection or hearing request may be claimed confidential by marking any part or all of that information as CBI. Information so marked will not be disclosed except in accordance with procedures set forth in 40 CFR part 2. A copy of the information that does not contain CBI must be submitted for

inclusion in the public record. Information not marked confidential may be disclosed publicly by EPA without prior notice.

Mail your written request to: Office of the Hearing Clerk (1900), Environmental Protection Agency, Ariel Rios Bldg., 1200 Pennsylvania Ave., NW., Washington, DC 20460. You may also deliver your request to the Office of the Hearing Clerk in Rm. C400, Waterside Mall, 401 M St., SW., Washington, DC 20460. The Office of the Hearing Clerk is open from 8 a.m. to 4 p.m., Monday through Friday, excluding legal holidays. The telephone number for the Office of the Hearing Clerk is (202) 260–4865.

2. *Tolerance fee payment.* If you file an objection or request a hearing, you must also pay the fee prescribed by 40 CFR 180.33(i) or request a waiver of that fee pursuant to 40 CFR 180.33(m). You must mail the fee to: EPA Headquarters Accounting Operations Branch, Office of Pesticide Programs, P.O. Box 360277M, Pittsburgh, PA 15251. Please identify the fee submission by labeling it "Tolerance Petition Fees."

EPA is authorized to waive any fee requirement "when in the judgement of the Administrator such a waiver or refund is equitable and not contrary to the purpose of this subsection." For additional information regarding the waiver of these fees, you may contact James Tompkins by phone at (703) 305–5697, by e-mail at tompkins.jim@epa.gov, or by mailing a request for information to Mr. Tompkins at Registration Division (7505C), Office of Pesticide Programs, Environmental Protection Agency, Ariel Rios Bldg., 1200 Pennsylvania Ave., NW., Washington, DC 20460.

If you would like to request a waiver of the tolerance objection fees, you must mail your request for such a waiver to: James Hollins, Information Resources and Services Division (7502C), Office of Pesticide Programs, Environmental Protection Agency, Ariel Rios Bldg., 1200 Pennsylvania Ave., NW., Washington, DC 20460.

3. *Copies for the Docket.* In addition to filing an objection or hearing request with the Hearing Clerk as described in Unit IX.A., you should also send a copy of your request to the PIRIB for its inclusion in the official record that is described in Unit I.B.2. Mail your copies, identified by docket number OPP–300997, to: Public Information and Records Integrity Branch, Information Resources and Services Division (7502C), Office of Pesticide Programs, Environmental Protection Agency, Ariel Rios Bldg., 1200 Pennsylvania Ave., NW., Washington, DC 20460. In person or by courier, bring a copy to the location of the PIRIB described in Unit I.B.2. You may also send an electronic copy of your request via e-mail to: opp-docket@epa.gov. Please use an ASCII file format and avoid the use of special characters and any form of encryption. Copies of electronic objections and hearing requests will also be accepted on disks in WordPerfect 6.1/8.0 file format or ASCII

file format. Do not include any CBI in your electronic copy. You may also submit an electronic copy of your request at many Federal Depository Libraries.

B. When Will the Agency Grant a Request for a Hearing?

A request for a hearing will be granted if the Administrator determines that the material submitted shows the following: There is a genuine and substantial issue of fact; there is a reasonable possibility that available evidence identified by the requestor would, if established resolve one or more of such issues in favor of the requestor, taking into account uncontested claims or facts to the contrary; and resolution of the factual issues(s) in the manner sought by the requestor would be adequate to justify the action requested (40 CFR 178.32).

X. Regulatory Assessment Requirements

This final rule establishes an exemption from the tolerance requirement under FFDCA section 408(d) in response to a petition submitted to the Agency. The Office of Management and Budget (OMB) has exempted these types of actions from review under Executive Order 12866, entitled *Regulatory Planning and Review* (58 FR 51735, October 4, 1993). This final rule does not contain any information collections subject to OMB approval under the Paperwork Reduction Act (PRA), 44 U.S.C. 3501 *et seq.*, or impose any enforceable duty or contain any unfunded mandate as described under Title II of the Unfunded Mandates Reform Act of 1995 (UMRA) (Public Law 104–4). Nor does it require any prior consultation as specified by Executive Order 13084, entitled *Consultation and Coordination with Indian Tribal Governments* (63 FR 27655, May 19, 1998); special considerations as required by Executive Order 12898, entitled *Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations* (59 FR 7629, February 16, 1994); or require OMB review or any Agency action under Executive Order 13045, entitled *Protection of Children from Environmental Health Risks and Safety Risks* (62 FR 19885, April 23, 1997). This action does not involve any technical standards that would require Agency consideration of voluntary consensus standards pursuant to section 12(d) of the National Technology Transfer and Advancement Act of 1995 (NTTAA), Public Law 104–113, section 12(d) (15 U.S.C. 272 note). Since tolerances and exemptions that are established on the basis of a petition under FFDCA section 408(d), such as the exemption in this final rule, do not require the issuance of a proposed rule, the requirements of the Regulatory Flexibility Act (RFA) (5 U.S.C. 601 *et seq.*) do not apply. In addition, the Agency has determined that this action will not have a substantial direct effect on States, on the relationship between the national government and the States, or on the distribution of power and responsibilities among the various levels of government, as specified in Executive Order 13132, entitled *Federalism* (64 FR 43255, August 10, 1999). Executive Order 13132 requires EPA to develop an accountable process to ensure "meaningful and timely input by State

and local officials in the development of regulatory policies that have federalism implications." "Policies that have federalism implications" is defined in the Executive Order to include regulations that have "substantial direct effects on the States, on the relationship between the national government and the States, or on the distribution of power and responsibilities among the various levels of government." This final rule directly regulates growers, food processors, food handlers and food retailers, not States. This action does not alter the relationships or distribution of power and responsibilities established by Congress in the preemption provisions of FFDCA section 408(n)(4).

XI. Submission to Congress and the Comptroller General

The Congressional Review Act, 5 U.S.C. 801 *et seq.*, as added by the Small Business Regulatory Enforcement Fairness Act of 1996, generally provides that before a rule may take effect, the agency promulgating the rule must submit a rule report, which includes a copy of the rule, to each House of the Congress and to the Comptroller General of the United States. EPA will submit a report containing this rule and other required information to the U.S. Senate, the U.S. House of Representatives, and the Comptroller General of the United States prior to publication of this final rule in the **Federal Register**. This final rule is not a "major rule" as defined by 5 U.S.C. 804(2).

List of Subjects in 40 CFR Part 180

Environmental protection, Administrative practice and procedure, Agricultural commodities, Pesticides and pests, Reporting and recordkeeping requirements.

Dated: JUNE 20 2000

JOSEPH J. MERENDA, JR.

Acting Deputy Director, Office of Pesticide Programs.

Therefore, 40 CFR chapter I is amended as follows:

PART 180—[AMENDED]

1. The authority citation for part 180 continues to read as follows:

Authority: 21 U.S.C. 321(q), 346(a) and 371.

2. Section 180.1209 is added to subpart D to read as follows:

§ 180.1209 Bacillus subtilus strain QST 713; exemption from the requirement of a tolerance.

An exemption from the requirement of a tolerance is established for residues of the microbial pesticide *Bacillus subtilus* strain QST 713 when used in or on all food commodities.

[FR Doc. 00–????? Filed ??–??–00; 8:45 am]

BILLING CODE 6560–50–F

April 7, 2000

→ Susanne FYI

Dr. Phil Hutton, Chief
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division (751C)
U.S. Environmental Protection Agency
Office of Pesticide Programs
401 M Street, S.W.
Washington, D.C. 20460

Re: Serenade™ WP Biofungicide

Dear Dr. Hutton:

My name is Kenton Kidd and I am the President of the California Apple Commission. The California Apple Commission promotes California apples by conducting marketing and advertising programs, providing information to assist the state's growers, and lobbying to protect the interests of the California apple industry. The California Apple Commission is involved in many programs to reduce the use of pesticides and find effective alternatives.

I am writing this letter to urge you and your staff to complete the review of the Serenade WP Biofungicide registration package. Control of fireblight on pears and apples in the U.S. is now at a critical moment due to questions regarding the availability of Blightban (Pseudomonas fluorescens) from PHT. Additionally, resistance to streptomycin sulfate makes these applications somewhat unpredictable and puts tremendous pressure on the only other registered bactericide product for fireblight control. Registration of Serenade WP will provide apple & pear growers with a critical tool for fireblight protection for this season if the registration arrives in time.

The advantages of making Serenade Biofungicide available to apple & pear growers are clear. Serenade is efficacious, can be applied with conventional application equipment, is compatible with other crop production products and provides relief in resistance management programs that all growers follow. In addition, Serenade is an environmentally friendly pesticide whose active ingredient is a ubiquitous microorganism found globally.

If you have any questions and would like to contact me, I can be reached at (559) 456-0900, or my email at kidd@calapple.com.

Sincerely yours,

Kenton Kidd

Kenton E. Kidd
President
California Apple Commission

c: Paul Heliker, Director, California Department of Pesticide Regulation
Jerry Campbell, Program Supervisor, Registrations Branch

California Apple Commission
4974 E. Clinton Way
Suite 125
Fresno, CA 93727-1520
Tel: 559/456-0900
Fax: 559/456-0125
www.calapple.com

G/word/chemical/serenade/microbial4700

Sam LeFore Fruit Farms, Inc.
54103 LeFore Road
Milton-Freewater,OR 97862
(541) 938-3528

March 26,2000

Dr. Phil Hutton, Chief
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division (751C)
U.S. Environmental Protection Agency
Office of Pesticide Programs
401 M Street S.W.
Washington, D.C. 20460

RE: Serenade WP Biofungicide

Dear Dr. Hutton:

My name is Sam LeFore. I am a fruit farmer in Milton-Freewater, Oregon. I grow mainly apples. I farm/manage approximately 400 acres of apples in the Milton-Freewater valley. I have been farming for more than 40 years.

I am writing this letter to urge you and your staff to complete the review of the Serenade WP Biofungicide registration package. Control of fire blight on apples in the U.S. is now at a critical moment due to questions regarding the availability of Blightban from PHT. Additionally, resistance to streptomycin sulfate makes these applications somewhat unpredictable and puts tremendous pressure on the only other registered bactericide product for fireblight control. Registration of Serenade WP will provide apple and pear growers with a critical tool for fire blight protection for this season of the registration arrives in time.

The advantages of making Serenade Biofungicide available to apple and pear growers are clear. Serenade is efficacious, can be applied with conventional application equipment, is compatible with other crop production products and provides relief in resistance management programs that all growers follow. In addition, Serenade is an environmentally friendly pesticide whose active ingredient is an ubuquitous microorganism found globally.

If you have question and would like to contact me, I can be reached by fax at (541) 938-3369 or the phone number listed above.

Sincerely,
Sam LeFore

March 6, 2000

Dr. Phil Hutton, Chief
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division (751C)
U. S. Environmental Protection Agency
Office of Pesticide Programs
401 M Street, S.W.
Washington, D.C. 20460

RE: Serenade WP Biofungicide

Dear Dr. Hutton:

My name is Alan Davis, and I represent Davis Orchards, Inc. Davis Orchards, Inc. grows 150 acres of apples in the Milton-Freewater, Oregon, area, and we support the Pesticide Environmental Stewardship program.

I am writing this letter to urge you and your staff to complete the review of the Serenade WP Biofungicide registration package. Control of fire blight on pears and apples in the U.S. is now at a critical moment due to questions regarding the availability of Blightban (Pseudomonas fluorescens) from PHT. Additionally, resistance to streptomycin sulfate makes these applications somewhat unpredictable and puts tremendous pressure on the only other registered bactericide product for fire blight control. Registration of Serenade WP will provide apple & pear growers with a critical tool for fire blight protection for this season if the registration arrives in time.

The advantages of making Serenade Biofungicide available to apple & pear growers are clear. Serenade is efficacious, can be applied with conventional application equipment, is compatible with other crop production products and provides relief in resistance management programs that all growers follow. In addition, Serenade is an environmentally friendly pesticide whose active ingredient is an ubiquitous microorganism found globally.

If you have any questions and would like to contact me, I can be reached at 541-938-7093.

Sincerely yours,

Alan Davis
Davis Orchards, Inc.
53285 Appleton Road
Milton-Freewater, OR 97862

Cc: Janet E. Fultz, Supervisor of Pesticide Registration and Certification, Oregon Department Of Agriculture
Dan Blevins, Pesticide/Fertilizer Specialist, Oregon Department of Agriculture, Pesticide Division
635 Capital Street NE
Salem, OR 97301-2532

Michele Knorr/DC/USEPA/US@EPA on 03/21/2000 12:10:54 PM

To: Susanne Cerrelli/DC/USEPA/US@EPA
cc:
Subject: Re: tolerance ex. for Qst 713 strain of B. subtilus -Reply -Reply

---

I concur on the revisions. Thank you for incorporating my comments.

Michele

CALIFORNIA
PEAR
Advisory Board

Wednesday, March 01, 2000

Dr. Phil Hutton, Chief
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division (751C)
U.S. Environmental Protection Agency
Office of Pesticide Programs
401 M Street, S.W.
Washington, D.C. 20460

RE: Serenade™ WP Biofungicide

Dear Dr. Hutton:

On Behalf of the California pear industry.

The California Pear Advisory Board (CPAB) is a state marketing order which allows pear growers to assess themselves to fund various industry programs. The Marketing order represents fresh and processed Bartlett pears produced in California. Our programs include research, pesticide advocacy, the dissemination of industry statistical, general, and consumer education information, and the promotion and marketing of California Bartlett pears.

CPAB is a charter member of EPA's Pesticide Environmental Stewardship Program and the California Department of Pesticide Regulation's Pest Management Alliance.

We are writing this letter to urge you and your staff to complete the review of the Serenade WP Biofungicide registration package.

Control of fire blight on pears in the United States is a major problem and has become a critical concern due to recent questions regarding the availability of the main blight control material - Blightban *(Pseudomonas fluorescens)* produced and distributed by Plant Health Technologies (J.R. Simplot). Additionally, resistance to streptomycin sulfate makes these applications somewhat unpredictable and puts tremendous pressure on the only other registered bactericide product for fireblight control (Oxytetracycline, *Mycoshield*).

1521 "I" Street • Sacramento, CA 95814~2016 • (916) 441~0432 • fax (916) 446~1063

The registration of Serenade WP will provide pear growers with a critical reduced risk tool for fire blight protection for this season if registration is completed on time.

The advantages of making Serenade Biofungicide available to pear growers are clear. Serenade is efficacious and can be applied with conventional application equipment. It is compatible with other crop production products and provides relief in the resistance management programs established in the pear industry in California. In addition, Serenade is an environmentally friendly pesticide whose active ingredient is a ubiquitous microorganism found globally.

Please contact us if you have any questions (916)-441-0432.

Sincerely yours,

Chris Zanobini
Executive Director
Email - chris @cpab.org

Mr. Bob McClain
Research Coordinator
email - bobcpab@aol.com

cc: Paul Helliker, Director, California Department of Pesticide Regulation
Jerry Campbell, DPR, Program Supervisor, Registrations Branch
Pat Cimino, Minor Crop Specialist, EPA, Office of Pesticide Programs

\\Bob\c\My Documents\2000 Pesticides\Seranade.doc

February 29, 2000

Dr. Phil Hutton, Chief
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division (751C)
U.S. Environmental Protection Agency
Office of Pesticide Programs
401 M Street, S.W.
Washington, D.C. 20460

RE: Serenade WP Biofungicide

Dear Dr. Hutton,

My name is Chris Frieders. I manage Esperanza Ranches which grows 400 acres of Bartlett pears and 70 acres of Bing cherries in Courtland California, in the Sacramento River Delta.

I cannot emphasize enough how important it is to the pear industry that you and your staff complete the review of the Serenade WP Biofungicide registration package. I have just learned from my supplier that the material Blightban (Pseudomonas fluorescens) from PHT, will not be available to use this season for the control of fire blight in pears. I can only make limited applications with streptomycin sulfate because of resistance problems. The other bactericide, terramycin, can only be used for part of the growing season due to pre-harvest restrictions. Registration of Serenade WP is desperately needed to provide a tool to combat fire blight in our pear orchards this year if the registration arrives in time.

The advantages of making Serenade Biofungicide available to apple and pear growers are clear. Serenade is efficacious, can be applied with congenital application equipment, is compatible with other crop production products and provides relief in resistance management programs that all growers follow. In addition, Serenade is an envrionmentally friendly pesticide whose active ingredient is an ubiquitous microorganism found globally.

If you have any questions please feel free to contact me at 916.775.1519
Sincerely yours,

Chris Frieders,
Esperanza Ranches, 200 Lambert Rd. Courtland Ca. 95615

cc; Paul Helliker, Director, California Department of Pesticide Regulation
Jerry Cambell, Program Supervisor, Registrations Branch

MID VALLEY AGRICULTURAL SERVICES INC.

P.O. Box 593
Linden, CA 95236
Phone (209) 931-9411
Fax (209) 931-0747

February 28, 2000

Dr. Phil Hutton

Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division (751C)
U.S. Environmental Protection Agency
Office of Pesticide Programs
401 M Street, S W.
Washington, D.C. 20406

RE: Serenade ™ WP Biofungicide

Dear Dr. Hutton

I am in charge of the technical services for Mid Valley Agricultural Services at Linden, California and Ripon Farm Service at Ripon California. . I provide technical services for 36 Pest Control Advisors. I evaluate the efficaciousness of new pesticides as they approach the market place. I must decide if the product in question will provide a benefit(s) not currently available to our clients. Mid Valley and Ripon are full service agricultural chemical and fertilizer outlets. They provide service to growers of grapes (wine), cherries, apples, pears, peaches, apricots, almonds, walnuts, chestnuts, asparagus, tomatoes, peppers, sugar beets, alfalfa, melons, pumpkins, corn, wheat, barley. Those are the main crops,

I wish to urge you and your staff to complete the review of the Serenade WP Biofungicide registration package. Control of fire blight on pears and apples in California and the U.S. is at a critical stage due to the questions about the availability of Blight Ban *(Pseudomonas fluorescens)* from PHT. Resistance to streptomycin sulfate makes its use unpredictable and puts tremendous pressure on the only other bactericide registered for use. Registration of Serenade WP will provide apple and pear growers with a critical tool for fire blight protection for this season if the registration arrives in time.

Serenade Biofungicide provides several advantages to growers. Serenade is efficacious; it can be applied by conventional equipment, its compatible with other crop protection products. The major benefit is it provides a resistance management tool for growers! Serenade is an environmentally friendly pesticide that does not add a chemical residue to the plant or soil.

If you have any questions and would like to contact me, my office number is (209) 931-9411 or my mobile is (209) 471-8544.

Sincerely,

Allan L James

Allan L. James Ph.D.
Technical Services, Mid Valley Ag Services

SUSAN Pgt for file.

February 24, 2000

Dr. Phil Hutton, Chief
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division (751C)
U.S. Environmental Protection Agency
Office of Pesticide Programs
401 M Street, S.W.
Washington, D.C. 20460

RE: Serenade™ WP Biofungicide

Dear Dr. Hutton:

I am an apple grower and also serve as president of the Mid-Valley Apple Association. Mid-Valley Apple Association is an organization of more than 150 apple growers in California.

I am writing this letter to urge you and your staff to complete the review of the Serenade WP Biofungicide registration package. Control of fire blight on pears and apples in the U.S. is now at a critical moment due to questions regarding the availability of Blightban *(Pseudomonas fluorescens)* from PHT. Additionally, resistance to streptomycin sulfate makes these applications somewhat unpredictable and puts tremendous pressure on the only other registered bactericide product for fireblight control. Registration of Serenade WP will provide apple & pear growers with a critical tool for fire blight protection for this season if the registration arrives in time.

The advantages of making Serenade Biofungicide available to apple & pear growers are clear. Serenade is efficacious, can be applied with conventional application equipment, is compatible with other crop production products and provides relief in resistance management programs that all growers follow. In addition, Serenade is an environmentally friendly pesticide whose active ingredient is an ubiquitous microorganism found globally.

If you have any questions and would like to contact me, I can be reached at (209) 334-3424 or the address below.

Sincerely yours,

Jeff J. Colombini
President

cc: Paul Helliker, Director, California Department of Pesticide Regulation
Jerry Campbell, Program Supervisor, Registrations Branch

Columbia Ag Research, Inc.

5601 Binns Hill Rd.
Hood River, OR 97031

Phone (541) 387-3052
Fax (541) 387-4428

December 31, 1999

Sent Via Fax 703-308-7026

Phil Hutton, Chief
Microbial Pesticide Branch
Biopesticides and Pollution Prevention Division
401 M Street, S.W.
Washington, D.C. 20480

RE: Serenade WP Biofungicide

Dear Mr. Hutton:

I am writing this letter to urge the U.S. EPA to finalize the registration of Serenade WP Biofungicide. AgraQuest has informed me that the Agency has concerns regarding possible negative effects to honeybees with field applications of Serenade. I am a private agricultural researcher which performs field trials on a contract basis. I have conducted nineteen separate trials on apples and pears during the 1998 and 1999 growing seasons. Trials were located throughout Oregon and Washington. Many applications were made during the flowering period when bees were actively working in the field. In all of the trials I conducted, I never observed any adverse effects of Serenade applications to honeybees.

Having Serenade available for use early in the 2000 season will be beneficial to the entire agricultural community. Serenade WP Biofungicide provides growers with a biological alternative to conventional chemicals, one that is both efficacious and environmentally friendly. Again, I urge you to expedite this registration so that Serenade will be available when it's needed.

Sincerely,

Vernon Fischer, Jr.
Director of Research

Address that Dr Fox requested beused.
12/22/99

Innovative natural product solutions for pest management

AgraQuest, Inc.

JENNIFER RYDER FOX, PHD
Director of Regulatory Affairs

1530 Drew Avenue
Davis, CA 95616
tel. 1.530. 750.0150, Ext. 296
fax. 1.530. 750.0153
jfox@agraquest.com
www.agraquest.com

Same address on file in REFs

12/99

From: Don J. Waddle

To: Janet Anderson
401 M. St. S.W.
Washingotn D. C. 20460

Recieved
DEC 15 1999
OPP/EPPD

Subject: Serenade Registration

Janet,

I am a fieldman for a local co-op and grape farmer here in Eastern Washington St.

Last summer I had the opportunity to visit filed trials in California on fungicides.

The purpose of this letter is for support of a full section 3 registration for the product Serenade bio-fungicide from Agra Quest, in a timely fashion. This product showed excellent performance, time and time again.

We need to have a product like this for:

- Resistance Management
- New, softer compound
- Short PHI
- Reduced risk to consumer and applicator

Please let me know if I can provide any assistance with the registration process for Serenade.

Thank you for attention,

Don J. Waddle

BLEYHL FARM SERVICE, INC.

Don Waddle
Field Consultant, Fertilizer Division

Bleyhl
"Owned by Local Farmers"

108 N. Birch • P.O. Box 100
Grandview, WA 98930
(509) 882-1225
1-800-645-4416
Fax: (509) 882-4208
Home: (509) 837-2319

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES
DEC 12 1999

MEMORANDUM

SUBJECT: BPPD Review of Product Chemistry, Manufacturing Process, Analysis of Samples, and Human Health Effect Data Submitted by AgraQuest, Inc. to Support Registration of Serenade WP [Submission# S554243; ID# 069592-L; DP Barcode# D252037; Chemical# 006479].

TO: Susanne Cerrelli (PM-90)
Regulatory Action Leader
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511C)

FROM: Michael T. Watson, Ph.D., Plant Pathologist
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511C)

and

Carl Etsitty, Microbiologist
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511C)

THROUGH: John L. Kough, Senior Scientist
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511C)

ACTION REQUESTED: To review Product Chemistry, Manufacturing Process, Analysis of Samples, and Human Health Effect data submitted by AgraQuest, Inc., to determine if it is adequate to support registration of Serenade WP.

*****THIS DOCUMENT CONTAINS FIFRA CONFIDENTIAL BUSINESS INFORMATION*****

NOTE: AgraQuest indicates that QST 713 Wettable Powder and Serenade Wettable Powder are the same products (E. M. Bellet to S. Cerrelli, 8-31-99).

DATA REVIEW RECORD

Active Ingredient:	*Bacillus subtilus* strain QST 713	
Product Name:	QST 713 Technical Powder	
Company Name:	AgraQuest, Inc.	
ID No:	069592	
Chemical Number:	006479	
Submission Number:	S554243	
DP Barcode:	D252037	
MRID No:	446517-01	Physical and Chemical Properties (151A-16)- Serenade WP
	446517-02	Analysis of Samples (151A-13) - Serenade WP
	446517-03	Storage Stability - Serenade WP
	446519-01	Physical and Chemical Properties (151A-16) - QST 713 TP
	445519-02	Lot Characterization - QST 713 TP
	446519-03	Storage Stability - QST 713 TP
	446519-04	Manufacturing Process (151A-11) - QST 713 TP
	446519-05	Sensitivity of Detection - QST 713 TP
	446519-06	Acute Oral Toxicity/Pathogenicity (152A-10) - QST 713 TP
	446519-07	Acute Dermal Toxicity/Pathogenicity (152A-11) - QST 713 TP
	446519-08	Acute Intravenous Toxicity/Pathogenicity (152A-13) - QST 713 TP
	446519-09	Acute Pulmonary Toxicity/Pathogenicity (152A-12) QST 713 TP
	446527-05	Acute Inhalation Toxicity (152A-12) - Serenade WP
	446646-01	Primary Dermal Irritation (152A-14) - QST 713 TP
	446646-02	Primary Eye Irritation (152A-14) - QST 713 TP
	446647-01	Acute Oral Toxicity (152A-10) - Serenade WP
	446647-02	Acute Dermal Toxicity (152A-11) - Serenade WP
	446647-03	Primary Dermal Irritation - Serenade WP
	446647-04	Primary Eye Irritation (152A-14) - Serenade WP
	446647-05	Delayed Contact Hypersensitivity - Serenade WP
	448923-01	Manufacturing Process (151A-11) - Serenade WP
	448944-01	Manufacturing Process (151A-11) - Serenade WP

BACKGROUND:

AgraQuest has submitted data to support registration of Serenade WP Biofungicide. Serenade WP contains *Bacillus subtilis* Strain QST 713 as its active ingredient. *Bacillus subtilis* is a rod-shaped, gram positive, aerobic, motile (peritrichous flagella) bacterium which is ubiquitous in nature. The organism is commonly found in various ecological niches including soil, water and air. The bacterium commonly produces proteases and other enzymes and because of this, strains of the bacterium are commonly used for industrial production of enzymes and other chemicals. The bacterium also can produce an endospore which allows the organism to endure extreme environmental conditions (e.g. heat, drought).

The genus *Bacillus* is a large, diverse genus of bacteria that includes species such as *thuringiensis, licheniformis, pumilis, cereus* and *anthracis*. Two of these species, *B. cereus* and *B. anthracis,* are known to be pathogenic to humans and animals. Because of this, the ability to differentiate species is extremely important. Biochemical tests and other tools exist which allow for the proper identification of the organism in question, such as *B. subtilis*.

B. subtilis is not a frank human pathogen, but has been isolated from human infections. However, such occurrences are typically limited to patients who are immuno-compromised because of some other condition or disease. In addition, the organism does produce the enzyme subtilisin which has been reported to produce allergenic or hypersensitivity reactions to individuals repeatedly exposed to the enzyme. However, OSHA has established standards for industrial settings (the only likely potential for repeated exposure) for exposure limits to workers.

Overall, *B. subtilis* produces only a very small potential risk to human health. The organism is not a frank pathogen, but is similar to numerous other opportunistic pathogens which are ubiquitous in nature. In all likelihood, individuals who may be sensitive to the organism have already been exposed to it, and risk of increased exposure is likely only to exist for pesticide applicators and manufactures of the product. These individuals should be equipped with adequate personal protection equipment.

The product itself is formulated to be applied as a spray for control of several plant pathogenic fungi. For application to plants (including: cucurbits, grapes, hops, leafy vegetables, peanuts, peppers, potatoes, strawberries, and tomatoes) and fruit trees (pome & stone fruits and nut trees), the recommended rate ranges between 3 and 10 lbs/acre and 1,000 ppm for mushrooms. The application rates and times vary depending upon plant host and fungus to be controlled.

DISCUSSION:

Review of the data submitted by AgraQuest for registration of Serenade WP Biofungicide indicates that the formulated product is not likely to create any human health concerns. The submission contains toxicity and pathogenicity testing of both the technical and end-use products. Based upon results of these tests, Serenade WP is not likely to cause adverse effects to humans who are exposed to the biofungicide. The enclosed studies describe appropriate quality control tests, which are incorporated into the manufacturing process, to insure that there are no detectable human pathogenic organisms present in the formulated product.

RECOMMENDATION:

SUPPLEMENTAL. Most of the data described and information submitted are acceptable to support registration of Serenade WP. However, some of the studies require further clarification, justification or additional information for them to be considered complete and acceptable. The submission can be upgraded to ACCEPTABLE with submission of adequate information/clarification for the deficiencies described below.

1. MRID# 4446517-03: Storage Stability - data for the 12 month storage stability of the end-use product has not been submitted. This data, along with clarification/justification of the plating technique description [pg. 7/20 "....allowed to stand before use"] should be provided.

2. MRID# 446519-03: Storage Stability - as mentioned for the end-use product, clarification/justification of the plating technique description [pg. 7/20 "....allowed to stand before use"] should be provided.
3. MRID# 446519-04: Manufacturing Process - the following information/clarification should be provided: the proposed active ingredient cfu range (upper/lower limit); a percent recovery error, based on sensitivity and limit of detection; additional broth production lot/batch analysis - 5 dry and 2; justification of [redacted] limit of detection [redacted]
4. MRID# 446527-05: Acute Inhalation - the test was performed with material which was diluted in water at a 25% w/w concentration. Therefore, the actual concentration of the test material was not determined. The analytical data which provides the actual concentration of the test material should be provided.
5. MRID# 448923-01: Manufacturing Process - additional information should be provided to identify the proposed range of active ingredients counts, percent recovery error data to support theoretical calculations based on lot analysis. In addition, a justification of the [redacted] limit of detection of [redacted] should be provided.
6. MRID# 448944-01: Manufacturing Process - additional information should be provided to identify the proposed active ingredient nominal concentration (with upper and lower limits), percent recovery error data to support theoretical calculations based on lot analysis. In addition, a justification of the [redacted] limit of detection of [redacted] should be provided.

SUMMARY OF DATA SUBMITTED:

446517-01 Physical and Chemical Properties [151A-16 (Serenade WP)]:
Serenade (*Bacillus subtilis* Strain QST 713) exists as a light brown, non-flammable, non-explodable powder, with an earthlike odor, and a bulk density of 30.0 lbs/ft^3 @ 20°C. Serenade is stable for at least one year at ambient storage storage conditions.
CLASSIFICATION: ACCEPTABLE

446517-02 Analysis of Samples [151A-13 (Serenade WP)]:
Colony morphologies and microscopic characteristics varied among different media, due to the temperatures, atmospheric conditions, and time variations. Otherwise, all five batches contained the MCPA at a concentration between 2.3×10^{10} and 4.7×10^{10} cfu/g (n=5), with no apparent microbial contamination.
CLASSIFICATION: ACCEPTABLE.

446517-03 Storage Stability [Serenade WP]:
Colony forming units are evaluated on TSA plates. The initial counts of the five received lot production of MCPA is 2.3×10^{10} to 4.7×10^{10} cfu/g (n=5), TSA plates. The packet is classified as SUPPLEMENTAL – may be upgraded to ACCEPTABLE with the submission of the final report (12 mon study), and clarification/justification of plating technique: "... allowed to stand before use" (7 of 20).
CLASSIFICATION: SUPPLEMENTAL – May be upgraded to ACCEPTABLE with the submission of the final report (12 mon study) and clarification/justification of plating technique: "... allowed to stand before use" (7 of 20).

446519-01 Physical and Chemical Properties [151A-16 (QST 713 TP)]:
QST 713 Technical (*Bacillus subtilis* Strain QST 713) exists as light brown, powder, non flammable, non explodable, with an earthlike odor, and a bulk density of 30.0 lbs/ft^3 @ 20°C; > 1 y storage as described in MRID 446517-03 (Storage Stability Data).
CLASSIFICATION: ACCEPTABLE

446519-02 Analysis of Samples [151A-13 (QST 713 TP)]:
Colony morphologies and microscopic characteristics varied among different media, due to the temperatures, atmospheric conditions, and time variations. Otherwise, all five production lots contained the MCPA at a concentration between of 2.6 x 10^6 and 2.2 x 10^{10} cfu/g (n=5) with no apparent microbial contamination. The moisture content was 4 to 6%.
CLASSIFICATION: ACCEPTABLE

446519-03 Storage Stability [QST 713 TP]:
Colony forming units are evaluated on TSA plates. The overall counts of the five received lot production of MCPA is 1.3 x 10^7 to 2.1 x 10^{10} cfu/g (n=5). The longevity of QST 713 technical will be stable after 12 mon storage at ambient temperature, with a ± 30% change. However, clarification should be provided to describe what is meant in the description of the of plating technique: "... allowed to stand before use" (pg. 7 of 18).
CLASSIFICATION: SUPPLEMENTAL. Can be upgraded to ACCEPTABLE with submission of a clarification for the deficiency described above.

446519-04 Manufacturing Process [151A-11 (QST 713 TP)]:
General steps to cultivation/harvesting, using the cultured seed of *Bacillus subtilis* QST 713 as an inoculum: [redacted]
CLASSIFICATION: SUPPLEMENTAL. The submission may be upgraded to ACCEPTABLE with submission of the following: Proposed active ingredient cfu range (upper/lower limit); Percent recovery error, based on sensitivity and limit of detection; Additional broth production lot/batch analysis - 5 dry and 2; Justification of [redacted] limit of detection [redacted]).

446519-05 Sensitivity of Detection (QST 713 TP):
QST 713 technical powder was tested for the sensitivity of detection to support

results of toxicity and pathogenicity testing in rats. Microbial recovery following addition of the test substance at a concentration of 4.3×10^{10} cfu/g, to lung and caecal tissue of male and female CD rats was determined. Test substance suspensions were mixed with tissue homogenates at levels of 10^2 and 10^4 cfu/ml and plated on trypticase soy agar (TSA) or TSA/Polymyxin B agar. Results indicate acceptable recovery for the lung tissue at both the 10^2 and 10^4 inoculum levels. For the caecal tissue, recovery was only acceptable for the 10^4 inoculum levels.
CLASSIFICATION: ACCEPTABLE. This is not a guideline requirement, but is acceptable data to support the registration of the product.

446519-06 Acute Oral Toxicity/Pathogenicity [152A-10 (QST 713 TP)]:
QST 713 Technical was administered orally to male and female rats at a dose of approximately 1.13×10^8 cfu/animal in a 1 ml volume. The test microbe was recovered on Days 0-7 from the stomach/small intestines, caecum, and feces of test animals dosed with the technical powder, but the organism was cleared before the Day 14 observation. In addition, the test microbe was recovered from the lungs, liver and mesenteric lymph nodes of one female rat (#302) on Day 0 and from the mesenteric lymph nodes of another female rat (#301), also on Day 0. No adverse clinical signs were observed, and gross necropsy did not reveal any abnormalities.
CLASSIFICATION: ACCEPTABLE

446519-07 Acute Dermal Toxicity/Pathogenicity [152A-11 (QST 713 TP)]:
Approximately 10% of the dorsal area fur of five male and five female rabbits was clipped and a dose of 2g/kg bodyweight was applied to the test site. The test site was covered with a 12.8 x 11.5 cm surgical dressing for 24 hours. Following the 24 hour exposure period, the residual test substance was removed with water moistened gauze pads. The animals were observed for clinical signs and/or skin irritation, "frequently" immediately following dosing and once per day for 13 days after removal of the wrapping. Edema, erythema and eschar formation was observed in all 10 rabbits, and multiple sores were observed in nine of the rabbits, following unwrapping. Necrosis was observed in some rabbits on Day 2, and in all rabbits by Day 4. Except for superficial skin flaking, all skin irritation effects were cleared in nine of the ten rabbits by Day 14. Rabbit #946 continued to exhibit edema, erythema, and eschar through Day 14. No rabbits died as a result of exposure to 2g/kg bodyweight of the test substance, therefore the LD_{50} of the test substance is greater than 2g/kg bodyweight.
CLASSIFICATION: ACCEPTABLE, TOXICITY CATEGORY III

446519-08 Acute Intravenous Toxicity/Pathogenicity (QST 713 TP):
A 1 g aliquot of the test substance (QST 713 TP) was diluted into 10 ml of water. The test substance was further diluted (1:215) to yield a concentration of 9.4×10^6 cfu in a volume of 0.5 ml for intravenous administration into rats. Control treatments included naive controls, shelf control and killed test substance groups. Rats, separated into appropriate groups, were sacrificed at 0, 7, 21, and 35 days post-

dosing. There was no mortality and no adverse clinical signs were observed in any of the rats dosed with the test substance. The test microbe was cleared from most organs by the Day 35 observation. However, low levels of the organism continued to be detected in the spleen and the liver after 35 days. The microbe was not detected in any of the animals in the control groups.
CLASSIFICATION: ACCEPTABLE

446519-09 Acute Pulmonary Toxicity/Pathogenicity [152A-12 (QST 713 TP)]:
Male and female rats were dosed intratracheally with QST 713 technical powder as a suspension in purified water at a concentration of 1.2×10^8 cfu/animal. Shelf control, killed test substance (KTG), and naive control (NC) rats groups were used as controls. Rats dosed with the test substance, as well as the KTG and NC rats were separated into groups (five rats/group/sex/day) for sacrifice at 0, 7, 21 and 35 days post dosing. There was no mortality as a result of the dosing, and other than a mottle lung parenchyma on Day 0, no other adverse effects were observed via gross necropsy. One male rat exhibited rough hair coat, but no other clinical signs were observed. Three female rats exhibited slight weight loss (one for two consecutive weeks), but the overall weight gain in all rats was similar. The test microbe was detectable (both pre- and post-heat treatments) in the lungs of the rats through Day 35, but at significantly reduced levels compared to Day 0. The organism was also detectable, post heat treatment, in the spleen, liver and kidneys of some of the animals through the Day 7 sacrifice. The organism was cleared in those organs in all animals by the Day 21 sacrifice.
CLASSIFICATION: ACCEPTABLE

446527-05 Acute Inhalation (Serenade WP) :
The four hour whole-body inhalation toxicity of QST 713 WP was evaluated in 10 Sprague-Dawley rats (5 male and 5 female). The rats received a time-weighted average aerosol concentration of 0.63 mg/L (the maximum maintainable concentration which gave a median aerodynamic particle size less than 4.0μm). Some clinical signs and weight loss were noted, but no mortality resulted from the dosing. A gross necropsy at the end of the 14 day observation period did not reveal any abnormalities. However, the test was performed with material diluted in water at a 25% w/w concentration, and therefore, an actual concentration was not determined. Despite this shortcoming, the aerodynamic particle size data for the dry aerosol indicate that approximately 64% of the particles are larger than 8.8μm in diameter and approximately 94% of the particles are larger than 5.1μm in diameter, with a Mass Median Aerodynamic Diameter (MMAD) of 10.5μm (GSD = 1.6). Normally, particles of this size pose a low risk of inhalation exposure. Therefore, although this report is classified as SUPPLEMENTAL, it will not affect the classification of the submission, based upon the low risk of exposure to the product.
CLASSIFICATION: SUPPLEMENTAL. Can be upgraded to ACCEPTABLE with submission of analytical data which indicates the actual concentration used in the dosing.

446646-01 Primary Dermal Irritation (QST 713 TP):
Approximately 500 mg of the test substance was applied to one intact site on the clipped dorsal trunk of each of six New Zealand White rabbits (3 male and 3 female). The test substance was moistened with 0.3 ml of saline, and covered with a gauze patch for a four hour exposure period. After 4 hours, the gauze was removed, and the residual test substance was removed using gauze moistened with water. The animals were examined for clinical and toxicological signs at 30 & 60 minutes, and 24, 48 and 72 hours (± 1 hour) after gauze removal. One animal lost a very small amount of weight (initial = 3.457 kg; final = 3.418 kg). Four animals showed slight erythema at the 30-60 minute evaluation, and three of these animals continued to show very slight erythema at the 24 hour observation. All of the these signs were resolved by the 48 hour observation.
CLASSIFICATION: ACCEPTABLE, TOXICITY CATEGORY IV

446646-02 Primary Eye Irritation [152A-12 (QST 713 TP)]:
A 0.1 ml (packed volume) sample of the test substance was placed into the conjunctival sac of the right eye of three male and three female New Zealand White rabbits. For each animal, the left eye of each served as an untreated control. The animals were examined at 1, 4, 24, 48, & 72 hours, as well as 4 days post- dosing and scored for ocular irritation. One animal (# 1883) lost a small amount of weight (304g) through the observation period. All six of the animals showed slight conjunctival effects through the 24 hour observation and three of the six animals continued to show slight conjunctival irritation through the 72 hour observation. In addition, 3/6, 2/6 and 1/6 of the animals showed slight iris effects through 24, 48 and 72 hours respectively. All of the signs cleared before the 96 hour observation.
CLASSIFICATION: ACCEPTABLE. TOXICITY CATEGORY III

446647-01 Acute Oral Toxicity [152A-10 (Serenade WP)]:
Five male and five female Sprague-Dawley Rats (Crl:CD®(SD)BR) were orally-dosed with 5000 mg/kg bodyweight of QST 173 wettable powder. Clinical observations were recorded at 1 and 4 hours post dosing, and daily through the 15 day observation period. None of the animals exhibited an abnormal clinical signs. Two female rats lost a small amount of weight (1 and 4 grams respectively) between the 8 and 15 day observation points, but both exhibited overall weight gain of 38 and 25 grams respectively. No abnormal effects were observed upon gross necropsy.
CLASSIFICATION: ACCEPTABLE, Toxicity Category IV

446647-02 Acute Dermal Toxicity [152A-11 (Serenade WP)]:
QST 713 wettable powder was applied to the prepared skin of 10 (five male and five female) New Zealand white rabbits at a concentration of 2000 mg/kg. Clinical observations were recorded at the time of unwrapping, and daily through the 15 day observation period. One of the animals (#1894) displayed an abnormal stance on days 3-6, but no other abnormal clinical signs were observed in any of the animals during the observation period. Also, there were signs of irritation and necrosis at the

application site in some of the animals (individual animals were not identified). All of the animals gained weight throughout the study and a gross necropsy did not reveal any abnormal effects of the treatment.
CLASSIFICATION: ACCEPTABLE, Toxicity Category III

446647-03 Primary Dermal Irritation (Serenade WP):
Five-hundred mg of QST 713 WP, moistened with 0.2 ml of saline, was applied to the clipped dorsal area of six New Zealand white rabbits. After a four hour exposure period, each rabbit was examined for signs of dermal irritation and scored according to Draize. All six animals showed very slight erythema at the 30-60 minute evaluations, with these signs resolving in each animal by the 72 hour observation. In addition, two animals showed very slight edema at the 30-60 minute (# 1888 & 1889) and at 24 and 48 hour observations (#1886 & 1889). No other clinical signs were noted through 72 hours, and no animals exhibited weight loss as a result of the dosing.
CLASSIFICATION: ACCEPTABLE

446647-04 Primary Eye Irritation [152A-14 (Serenade WP)]:
Approximately 100 mg (0.1 ml packed volume) of QST 713 WP was placed into the right eye of six (three male and three female) New Zealand white rabbits. The eyes of each animal were examined at 1, 24, 48, and 72 hours post dosing. All animals exhibited slight to moderate irritation of the conjunctivae (redness, chemosis and/or discharge) at the 1 hour observation. Slight redness persisted in four animals at the 24 hour observation and in three animals at the 48 hour observation. All of the irritation signs were resolved before the 72 hour observation.
CLASSIFICATION: ACCEPTABLE, Toxicity Category IV

446647-05 Delayed Contact Hypersensitivity (Serenade WP):
The purpose of this study was to determine if the test material, QST 713 WP, has the potential to elicit a delayed dermal contact hypersensitivity response in guinea pigs. The positive control group induced and challenged with DNCB exhibited the expected responses to provide validation of the test methods. Prior to the experimental initiation, the irritation potential of the test material was determined by a dose range study, and based upon the results of this study, the test article was dosed at 100%. Induction with the test material, QST 713 WP, did elicit a very mild delayed contact hypersensitivity response in guinea pigs which were challenged and rechallenged with the test material.
CLASSIFICATION: ACCEPTABLE. This product should be labeled as a potential dermal sensitizer.

448923-01 Manufacturing Process [151A-11(Serenade)]:

[redacted]

[redacted]

CLASSIFICATION: SUPPLEMENTAL – May be upgraded to ACCEPTABLE, with submission of the following: Proposed range of AI counts; Percent recovery error data to support theoretical calculations; Justification of [redacted] limit of detection [redacted]

448944-01 Manufacturing Process [151A-11 (Serenade WP)]:

[redacted]

CLASSIFICATION: SUPPLEMENTAL – May be upgraded to ACCEPTABLE, with submission of the following: Proposed AI nominal concentration, with upper and lower limits; Percent recovery error data to support theoretical calculations; Justification of [redacted] limit of detection [redacted]

Manufacturing process information may be entitled to confidential treatment

DATA EVALUATION REPORT

Reviewed by: Carl Etsitty, M.S., Microbiologist

Secondary Reviewer: John L. Kough, Ph.D., Senior Scientist

STUDY TYPE:	Color (OPPTS 830.6302), Physical State (OPPTS 830.6303), Odor (OPPTS 830.6304), pH (OPPTS 830.7000), Density/Relative Density/Bulk Density (OPPTS 885.7300)
MRID NO:	446517-01
TEST MATERIAL:	Serenade™ WP (*Bacillus subtilis* QST 713)
PROJECT NO:	None Assigned
SPONSOR:	AgraQuest, Inc., Davis, CA 95616
TESTING FACILITY:	AgraQuest, Inc., Davis, CA 95616
TITLE OF REPORT:	Product Chemistry for Serenade™ WP
AUTHOR(S):	E.M. Bellet, Ph.D.
STUDY COMPLETED:	August 26, 1998
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	Serenade exists as a light brown, non flammable, non explodable powder, with an earthlike odor, and a bulk density of 30.0 lbs/ft^3 @ 20°C; > 1 y storage
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN

Test Material: Serenade™ WP, active ingredient is *Bacillus subtilis* Strain QST 713

Methods:

Color: Light brown powder.

Physical State:

From MRID 446517-04, pp 16 of 52 to 19 of 52, the physical state was determined to be a powder form (e.g., wettable powder).

Bulk Density:

From MRID 446517-04, pp 21 of 52, the bulk density determination was described: A known bulk density volume cup was leveled with WP powder, after it was screened (≥ 10 mesh). WP minus the cup weight was recorded in grams/cup volume, and converted to lb/ft^2 (e.g., gram wt of sample ÷ cup volume x 62.428 = lb/ft^2).

Stability and Storage Stability:

From MRID 446517-03, the stability and storage stability were described: Five production lots (e.g, 32-38-2B, 32-38-3B, 32-38-4B, 32-38-5B, and 32-38-6B) were received on June 3, 1998, in 'ziploc' bags at ambient temperature, and stored (21-23 °C). The received samples will be evaluated in intervals (e.g., initial, 3, 6 and 12 mon), as a representation of a warehouse storage. Temperature and relative humidity (RH) was (will be) recorded.

One gram of 10 gram samples was removed from the received container. It was (will be) appropriately diluted and plated (0.1 mL/plate). The test substance was incubated 24 h at 35 °C, using Blood Agar (BA), Trypticase Soy Agar (TSA), Sabouraud Dextose Agar (SDA), and MacConkey Agar (MCA) plates. After 24 h, the plates were individually counted for colonies.

Results:

Physical/Chemical Properties:

	Serenade™ WP *Bacillus subtilis* Strain QST 713
Color	Light brown
Physical State	Powder
Odor	Earthlike
Melting Point	N.A.
Boiling Point	N.A.
Density/Specific Gravity (20°C)	45 lbs/ft^3
Solubility	N.A.
Vapor Pressure	N.A.
Dissociation Constant	N.A.
Oct./Water Part. Coeff.	N.A.
pH	N.A.
Stability	Stable
Oxid./Red.	N.A.
Flammability	Non-flammable
Explodability	Non-explodable
Storage Stability	Greater than 1 y
Viscosity	N.A.
Miscibility	N.A.

DISCUSSION:

As described in MRID 446517-03 (Storage Stability Data), the 1 y and greater shelf-life were based on an interim report, and the evaluation is still in progress.

DATA EVALUATION REPORT

Reviewed by: Carl Etsitty, M.S., Microbiologist

Secondary Reviewer: John L. Kough, Ph.D., Senior Scientist

STUDY TYPE:	Analysis of Samples (OPPTS 885.1400)
MRID NO:	446517-02
TEST MATERIAL:	Serenade™ WP (*Bacillus subtilis* Strain QST 713)
PROJECT NO:	L08726 SN8
SPONSOR:	AgraQuest, Inc., Davis, CA 95616
TESTING FACILITY:	IIT Research Institute, Life Sciences Operation, Chicago, ILL
TITLE OF REPORT:	Lot Characterization of QST 713 Strain of Dried *Bacillus subtilis* with Residual Fermentation Media Identified as QST 713 WP
AUTHOR(S):	Bruce A. Gingras, Ph.D.
GOOD LABORATORY PRACTICE:	GLP Compliant
STUDY COMPLETED:	July 1998
CONCLUSION:	All five batches is microbial pure with MCPA, with a range of 2.3 x 10^{10} to a high of 4.7 x 10^{10} cfu/g (n=5).
CLASSIFICATION:	ACCEPTABLE

ANALYSIS OF SAMPLES

I. STUDY DESIGN

Test Material: Serenade™ WP, active ingredient is *Bacillus subtilis* Strain QST 713

Method: Five production batches (e.g, 32-38-2B, 32-38-3B, 32-38-4B, 32-38-5B, and 32-38-6B) were quantified and characterized. Of the received production batches (microbial pest control agent – MCPA), one gram MCPA was tested, and appropriately diluted and plated (50 μl/plate). The test substance was evaluated at three temperatures: 35°C, aerobically for 2 d, anaerobically at 35°C for 8 d, and room temperature for 3 d, using Blood Agar (BA), Trypticase Soy Agar (TSA), Sabouraud Dextose Agar (SDA), and MacConkey Agar (MCA) plates in duplicates.

These plates were characterized by examining for colonial morphology, and gram staining for microscopic characteristics. Additionally, 0.1 mL aliquots were removed and plated in duplicate on TSA, BA, SDA and MCA plates, for 24 h at 35°C, to determine CFU.

Results: All five batches were microbially pure with MCPA, with a range of 2.3×10^{10} to a high of 4.7×10^{10} cfu/g (n=5), on TSA. Three (32-38-4B, 32-38-5B and 32-38-6B) of 5 batches had gram-positive bacterial growth, under anaerobic conditions, on BA.

Colony Forming Unit Data for Serenade™ WP, active ingredient is *Bacillus subtilis* Strain QST 713 Production Batches					
	32-38-2B	32-38-3B	32-38-4B	32-38-5B	32-38-6B
TSA Plates	221 CFU	219 CFU	234 CFU	45 CFU	238 CFU
	280 CFU	242 CFU	256 CFU	49 CFU	284 CFU
Titer (CFU/g)	2.5×10^{10}	2.3×10^{10}	2.5×10^{10}	4.7×10^{10}	2.6×10^{10}
MCA Plates	0	0	0	0	0
	0	0	0	0	0
Titer (CFU/g)	n/a	n/a	n/a	n/a	n/a
SDA Plates	26 CFU	81 CFU	87 CFU	60 CFU	27 CFU
	32 CFU	55 CFU	54 CFU	73 CFU	32 CFU
Titer (CFU/g)	2.9×10^{8}	6.8×10^{8}	7.1×10^{8}	6.7×10^{8}	3.0×10^{8}
BA Plates	198 CFU	134 CFU	268 CFU	37 CFU	178 CFU
	204 CFU	162 CFU	242 CFU	39 CFU	170 CFU
Titer (CFU/g)	2.0×10^{10}	1.5×10^{10}	2.6×10^{10}	3.8×10^{10}	1.7×10^{10}

II DISCUSSION

Colony morphologies and microscopic characteristics varied among different media, due to the temperatures, atmospheric conditions, and time variations. Otherwise, all five batches were microbial pure with MCPA, with a range of 2.3×10^{10} to a high of 4.7×10^{10} cfu/g (n=5). The packet classification is ACCEPTABLE.

DATA EVALUATION REPORT

Reviewed by: Carl Etsitty, M.S., Microbiologist

Secondary Reviewer: John L. Kough, Ph.D., Senior Scientist

STUDY TYPE:	Storage Stability Data (OPPTS 885.2400)
MRID NO:	446517-03
TEST MATERIAL:	Serenade™ WP (*Bacillus subtilis* Strain QST 713)
PROJECT NO:	L08726 SN9
SPONSOR:	AgraQuest, Inc., Davis, CA 95616
TESTING FACILITY:	IIT Research Institute, Life Sciences Operation, Chicago, ILL
TITLE OF REPORT:	Storage Stability of QST 713 Strain of Dried *Bacillus subtilis* with Residual Fermentation Media Identified as QST 713 WP
AUTHOR(S):	Bruce A. Gingras, Ph.D.
STUDY COMPLETED:	July 1998 (Interim Report)
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	Initial counts of the five received lot production of MCPA is 2.3 x 10^{10} to 4.7 x 10^{10} cfu/g (n=5), on TSA plates.
CLASSIFICATION:	SUPPLEMENTAL – May be upgraded to ACCEPTABLE with the submission of the final report (12 mon study) and clarification/justification of plating technique: "... allowed to stand before use" (7 of 20).

ANALYSIS OF SAMPLES

I. STUDY DESIGN

Test Material: Serenade™ WP, active ingredient is *Bacillus subtilis* Strain QST 713

Method: Five production lots (e.g, 32-38-2B, 32-38-3B, 32-38-4B, 32-38-5B, and 32-38-6B) were received on June 3, 1998, in 'ziploc' bags at ambient temperature, and stored (21-23°C). The received samples will be evaluated in intervals (e.g., initial, 3, 6 and

12 mon), as a representation of a warehouse storage. Temperature and relative humidity (RH) was (will be) recorded.

One gram of 10 gram samples was removed from the received container. It was (will be) appropriately diluted and plated (0.1 mL/plate). The test substance was incubated 24 h at 35°C, using Blood Agar (BA), Trypticase Soy Agar (TSA), Sabouraud Dextose Agar (SDA), and MacConkey Agar (MCA) plates. After 24 h, the plates were individually counted for colonies.

Number of Test Substance Lots Assayed				
Media	Initial	3 mon	6 mon	12 mon
TSA	5	5	5	5
BA	5	5	5	5
SDA	5	5	5	5
MCA	5	5	5	5

Results: The initial colony forming units (cfu) of QST 713 WP was ranged from 2.3 x 10^{10} to a high of 4.7 x 10^{10} cfu/g (n=5), with a max of 25°C and 80% RH, and a min of 21°C and 52% RH.

Storage Stability of QST 713 WP, Containing a Strain of *Bacillus subtilis*.: Lots Test Substance Titer [Colony forming Units (cfu/g)]: Initial					
	32-38-2B	32-38-3B	32-38-4B	32-38-5B	32-38-6B
TSA Plates	$2.5x10^{10}$	$2.3x10^{10}$	$2.5x10^{10}$	$4.7x10^{10}$	$2.6x10^{10}$
MCA Plates	0	0	0	0	0
SDA Plates	2.9 x 10^{8}	$6.8x10^{8}$	$7.1x10^{8}$	$6.7x10^{8}$	$3.0x10^{8}$
BA Plates	2.0 x 10^{10}	$1.5x10^{10}$	$2.6x10^{10}$	$3.8x10^{10}$	$1.7x10^{10}$

II DISCUSSION

Colony forming units are evaluated on TSA plates. The initial counts of the five received lot production of MCPA is 2.3 x 10^{10} to 4.7 x 10^{10} cfu/g (n=5), TSA plates. The packet is classified as SUPPLEMENTAL – may be upgraded to ACCEPTABLE with the submission of the final report (12 mon study), and clarification/justification of plating technique: "... allowed to stand before use" (7 of 20).

Manufacturing process information may be entitled to confidential treatment

DATA EVALUATION REPORT

Reviewed by: Carl Etsitty, Microbiologist

Secondary Reviewer: John L. Kough, Ph.D., Senior Scientist

STUDY TYPE:	Manufacturing Process (OPPTS 885.1200)
MRID NO:	446519-04
TEST MATERIAL:	QST 713 Technical or Technical Powder (TP) (*Bacillus subtilis* Strain QST 713)
PROJECT NO:	None Assigned
SPONSOR:	AgraQuest, Inc., Davis, CA 95616
TESTING FACILITY:	AgraQuest, Inc., Davis, CA 95616
TITLE OF REPORT:	Manufacturing and Analytical Data for QST 713 Technical
AUTHOR(S):	Laura Cunningham Hilbig, and E.M. Bellet, Ph.D.
STUDY COMPLETED:	None Assigned
GOOD LABORATORY PRACTICE:	Non GLP Compliant
CONCLUSION:	General steps to cultivation/harvesting, using the cultured seed of *Bacillus subtilis* QST 713 as an inoculum: [redacted]
CLASSIFICATION:	SUPPLEMENTAL – May be upgraded to ACCEPTABLE with the following submissions: Proposed AI cfu range (upper/lower limit); Percent recovery error, based on sensitivity and limit of detection, based on lot analysis; Provide 5 dry and 2 additional broth production lot/batch analysis; Justification of [redacted] limit of detection [redacted]

CONTAINS CONFIDENTIAL BUSINESS INFORMATION

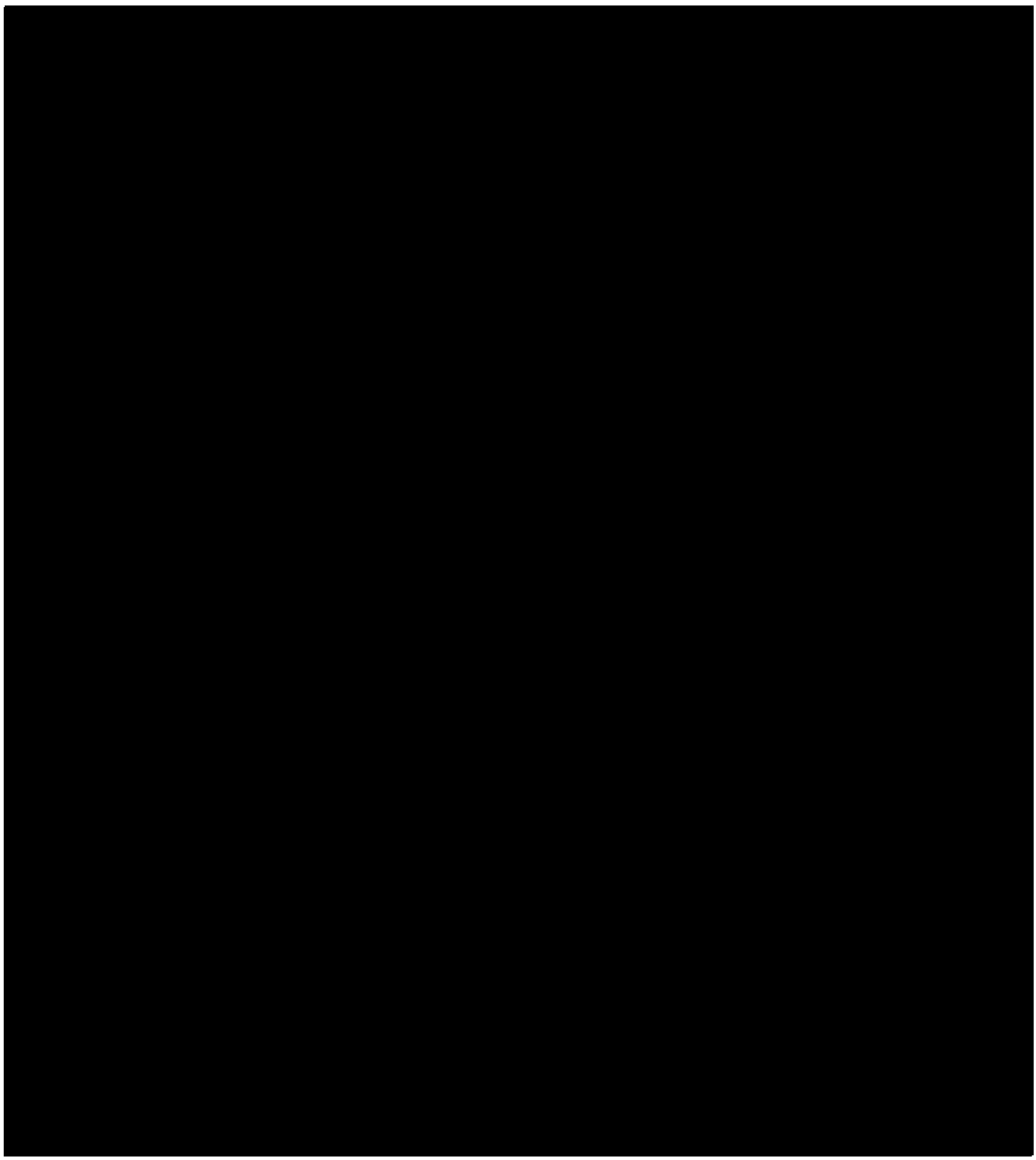
NOTE: After section 1.2.7 and 1.2.9, proceed onto appropriate End Use Manufacturing Process.

Manufacturing process information may be entitled to confidential treatment

1 **STUDY DESIGN**

1.1 Test Material: QST 713 Technical, active ingredient is *Bacillus subtilis* Strain QST 713

1.2 **MANUFACTURING PROCESS:**



Pages 50-53 - *Manufacturing process information may be entitled to confidential treatment*

2 DISCUSSION

[redacted]

3 COMMENT

[redacted]. Therefore, the packet is SUPPLEMENTAL, and may be up graded to ACCEPTABLE with the following submissions: Proposed AI cfu range (upper/lower limit); Percent recovery error, based on sensitivity and limit of detection, based on lot/batch analysis; Provide 5 dry and 2 additional broth production lot/batch analysis; Justification of [redacted] limit of detection [redacted].

DATA EVALUATION REPORT

Reviewed by: Carl Etsitty, M.S., Microbiologist

Secondary Reviewer: John L. Kough, Senior Scientist, Ph.D.

STUDY TYPE:	Color (OPPTS 830.6302), Physical State (OPPTS 830.6303), Odor (OPPTS 830.6304), pH (OPPTS 830.7000), Density/Relative Density/Bulk Density (OPPTS 885.7300)
MRID NO:	446519-01
TEST MATERIAL:	QST 713 Technical (*Bacillus subtilis* Strain QST 713)
PROJECT NO:	None Assigned
SPONSOR:	AgraQuest, Inc., Davis, CA 95616
TESTING FACILITY:	AgraQuest, Inc., Davis, CA 95616
TITLE OF REPORT:	Product Chemistry for QST 713 Technical
AUTHOR(S):	E.M. Bellet, Ph.D.
STUDY COMPLETED:	August 25, 1998
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	QST 713 Technical (*Bacillus subtilis* Strain QST 713) exists as light brown, non flammable, non explodable, powder, with an earthlike odor, and a bulk density of 30.0 lbs/ft^3 @ 20°C; > 1 y storage
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN

Test Material: Serenade™ WP, active ingredient is *Bacillus subtilis* Strain QST 713

Methods:

Color: From MRID 446517-02, pp 6 of 21 describes TP as light brown.

Physical State:

From MRID 446517-04, pp 16 of 52 to 19 of 52, the physical state was determined to be a powder form (e.g., wettable powder).

Bulk Density:

From MRID 446517-04, pp 21 of 52, the bulk density determination was described: A known bulk density volume cup was leveled with WP powder, after it was screened (≥ 10 mesh). WP minus the cup weight was recorded in grams/cup volume, and converted to lb/ft^2 (e.g., gram wt of sample ÷ cup volume x 62.428 = lb/ft^2).

Stability and Storage Stability:

From MRID 446517-03, the stability and storage stability were described: Five production lots (e.g, 32-38-2B, 32-38-3B, 32-38-4B, 32-38-5B, and 32-38-6B) were received on June 3, 1998, in 'ziploc' bags at ambient temperature, and stored (21-23 °C). The received samples will be evaluated in intervals (e.g., initial, 3, 6 and 12 mon), as a representation of a warehouse storage. Temperature and relative humidity (RH) was (will be) recorded.

One gram of 10 gram samples was removed from the received container. It was (will be) appropriately diluted and plated (0.1 mL/plate). The test substance was incubated 24 h at 35 °C, using Blood Agar (BA), Trypticase Soy Agar (TSA), Sabouraud Dextose Agar (SDA), and MacConkey Agar (MCA) plates. After 24 h, the plates were individually counted for colonies.

Results:

Physical/Chemical Properties:

	Serenade™ WP *Bacillus subtilis* Strain QST 713
Color	Light brown
Physical State	Powder
Odor	Earthlike
Melting Point	N.A.
Boiling Point	N.A.
Density/Specific Gravity (20°C)	30 lbs/ft^3
Solubility	N.A.
Vapor Pressure	N.A.
Dissociation Constant	N.A.
Oct./Water Part. Coeff.	N.A.
pH	N.A.
Stability	Stable
Oxid./Red.	N.A.
Flammability	Non-flammable
Explodability	Non-explodable
Storage Stability	Greater than 1 y
Viscosity	N.A.
Miscibility	N.A.

DATA EVALUATION REPORT

Reviewed by: Carl Etsitty, M.S., Microbiologist

Secondary Reviewer: John L. Kough, Ph.D., Senior Scientist

STUDY TYPE:	Analysis of Samples (OPPTS 885.1400)
MRID NO:	446519-02
TEST MATERIAL:	QST 713 Technical (*Bacillus subtilis* Strain QST 713)
PROJECT NO:	L08726 SN1
SPONSOR:	AgraQuest, Inc., Davis, CA 95616
TESTING FACILITY:	IIT Research Institute, Life Sciences Operation, Chicago, ILL
TITLE OF REPORT:	Lot Characterization of *Bacillus subtilis* Strain QST 713
AUTHOR(S):	Bruce A. Gingras, Ph.D.
GOOD LABORATORY PRACTICE:	GLP Compliant
STUDY COMPLETED:	August 1998
CONCLUSION:	All five production lots is microbial pure with MCPA, with a range, on different media, of 2.6 x 10^6 to a high of 2.2 x 10^{10} cfu/g (n=5); moisture content: 4-6%.
CLASSIFICATION:	ACCEPTABLE

ANALYSIS OF SAMPLES

I. STUDY DESIGN

Test Material: QST 713 Technical, active ingredient is *Bacillus subtilis* Strain QST 713

Method: Five production lots (e.g, 812-0910, 812-0911, 8AQ07C1, 8AQ10C2, and 8AQ11C2) were quantified and characterized. Of the received production lots (microbial pest control agent – MCPA), one gram MPCA was tested, and appropriately diluted and plated (50 μl/plate), in duplicates.

The test substance was evaluated at three temperatures: aerobically at 35°C for 24 h, anaerobically at 35°C for 7 d, and room temperature for 4 d, using Blood Agar (BA), Trypticase Soy Agar (TSA), Sabouraud Dextose Agar (SDA), and MacConkey

Agar (MCA) plates in duplicates. Aerobic plates incubated at 35 °C are removed and evaluated after 24 h. These plates were characterized by examining for colonial morphology, gram staining for microscopic characteristics. Additionally, aliquots for titers were serially diluted and enumerated by removing a 0.1 mL aliquots, and plated on TSA, BA, SDA and MCA plates, for 24 to 41 h at 35 °C, to determine CFU.

Results: All five batches were microbial pure with MCPA, with a range, on different media, of 2.6 x 10^6 to a high of 2.2 x 10^{10} cfu/g (n=5); moisture content: 4-6%.

Colony Forming Unit (cfu) Data for QST 713 Technical					
	812-0910	812-0911	8AQ07C1	8AQ10C2	8AQ11C2
TSA Plates	224 CFU	173 CFU	190 CFU	75 CFU	92 CFU
	185 CFU	163 CFU	202 CFU	81 CFU	105 CFU
Titer (CFU/g)	$2.1x10^{10}$	$1.7x10^{10}$	$2.0x10^{10}$	$7.8x10^7$	$9.9x10^6$
MCA Plates	0	0	0	0	0
	0	0	0	0	0
Titer (CFU/g)	n/a	n/a	n/a	n/a	n/a
SDA Plates	133 CFU	153 CFU	19 CFU	30 CFU	28 CFU
	84 CFU	182 CFU	21 CFU	24 CFU	23 CFU
Titer (CFU/g)	1.1 x 10^9	$1.7x10^{10}$	$2.0x10^9$	$2.7x10^7$	$2.6x10^6$
BA Plates	147 CFU	131 CFU	211 CFU	112 CFU	178 CFU
	149 CFU	119 CFU	220 CFU	104 CFU	152 CFU
Titer (CFU/g)	1.5 x 10^{10}	$1.3x10^{10}$	$2.2x10^{10}$	$1.1x10^8$	$1.7x10^7$
Moisture Content (%)	4.44	5.57	4.22	4.32	6.00

Morphology: Colony characteristics varied with different media. Colony forms ranged from irregular-regular, diameters are 2-6 mm, colors are light cream (brownish) to cream (white), with opaque densities. Furthermore, they are flat-raised to raised, and surfaces are either smooth, or slightly rough on the edge, or rough, and showing a margin of undulate, or lobate.

II DISCUSSION

Colony morphologies and microscopic characteristics varied among different media, due to the temperatures, atmospheric conditions, and time variations. Otherwise, all five production lots were

microbial pure with MCPA, with a range, on different media, of 2.6 x 10^6 to a high of 2.2 x 10^{10} cfu/g (n=5); moisture content: 4 to 6%. The packet classification is ACCEPTABLE.

DATA EVALUATION REPORT

Reviewed by: Carl Etsitty, M.S., Microbiologist

Secondary Reviewer: John L. Kough, Ph.D., Senior Scientist

STUDY TYPE:	Storage Stability Data (OPPTS 885.2400)
MRID NO:	446519-03, 449071-01
TEST MATERIAL:	QST 713 Technical (*Bacillus subtilis* Strain QST 713)
PROJECT NO:	L08726 SN2
SPONSOR:	AgraQuest, Inc., Davis, CA 95616
TESTING FACILITY:	IIT Research Institute, Life Sciences Operation, Chicago, ILL
TITLE OF REPORT:	Storage Stability of Technical QST 713
AUTHOR(S):	Bruce A. Gingras, Ph.D.
GOOD LABORATORY PRACTICE:	GLP Compliant
STUDY COMPLETED:	July 1999 (Final Report)
CONCLUSION:	Initial and final (12 mon) counts of the five received lot production of MCPA is 9.9×10^6 to 2.0×10^{10} cfu/g (n=5), and 1.3×10^7 to 2.1×10^{10} cfu/g (n=5), with a ±30% change, when stored at ambient temperature.
CLASSIFICATION:	SUPPLEMENTARY – May be upgraded to ACCEPTABLE, with the submission of a clarification or justification of plating technique: "... allowed to stand before use" (7 of 18).

ANALYSIS OF SAMPLES

I. STUDY DESIGN

Test Material: QST 713 Technical, active ingredient is *Bacillus subtilis* Strain QST 713, is a light brown spray dried powder, plus fermentation broth.

Method: Five production lots (e.g, 812-0910, 812-0911, 8AQ07C1, 8AQ10C2, and 8AQ11C2) were received on April 14 or 16, 1998, in 50cc centrifuge tubes at ambient temperature, and stored (21-24°C). The received samples will be evaluated

in intervals (e.g., initial, 3, 6 and 12 mon), as a representation of a warehouse storage. Temperature and relative humidity (RH) was recorded.

One gram of 10 gram samples was taken from the provided foil-lined pouches[1], which was removed from the received container. It was appropriately diluted and plated (0.1 mL/plate), in duplicates. The test substance was incubated 24 h at 35 °C, using Blood Agar (BA), Trypticase Soy Agar (TSA), Sabouraud Dextose Agar (SDA), and MacConkey Agar (MCA) plates. After 24 h, the plates were individually counted for colonies. The following table shows the design of number of test substance lots assayed:

Number of Test Substance Lots Assayed				
Media	Initial	3 mon	6 mon	12 mon
TSA	5	5	5	5
BA	5	5	5	5
SDA	5	5	5	5
MCA	5	5	5	5

Results: The overall 12 mon max/min colony forming units (cfu) of QST 713 Technical was ranged from 1.3 x 10^7 to a high of 2.1 x 10^{10} cfu/g (n=5), with a max of 32 °C and 80% RH, and a min of 18 °C and 14% RH, on TSA medium.

Storage Stability of QST 713 Technical, Containing a Strain of *Bacillus subtilis*.: Lots Test Substance Titer [Colony forming Units (cfu/g)]: Initial					
	812-0910	812-0911	8AQ07C1	8AQ10C2	8AQ11C2
TSA Plates	$2.0x10^{10}$	$1.7x10^{10}$	$2.0x10^{10}$	$7.8x10^{7}$	$9.9x10^{7}$
MCA Plates	0	0	0	0	0
SDA Plates	$1.1x10^{9}$	$1.7x10^{10}$	$2.0x10^{9}$	$2.7x10^{7}$	$2.6x10^{6}$
BA Plates	$1.5x10^{10}$	$1.3x10^{10}$	$2.2x10^{10}$	$1.1x10^{8}$	$1.7x10^{7}$

Storage Stability of QST 713 Technical, Containing a Strain of *Bacillus subtilis*.: Lots Test Substance Titer [Colony forming Units (cfu/g)]: 3 mon					
	812-0910	812-0911	8AQ07C1	8AQ10C2	8AQ11C2
TSA Plates	$8.1x10^{9}$	$8.4x10^{9}$	$1.8x10^{10}$	$8.6x10^{7}$	$1.3x10^{6}$
MCA Plates	0	0	0	0	0

[1]6x9"/ 21# paper/ 7# PE/ 48 ga. Met Pet/ 10# PE/ 2 Mil LLDPE, Graphic Packing Corp

SDA Plates	$6.6x10^8$	$4.7x10^9$	$7.9x10^8$	$3.5x10^7$	$1.7x10^6$
BA Plates	$1.7x10^{10}$	$1.5x10^{10}$	$2.7x10^{10}$	$1.5x10^8$	$1.9x10^7$

Storage Stability of QST 713 Technical, Containing a Strain of *Bacillus subtilis*.: Lots Test Substance Titer [Colony forming Units (cfu/g)]: 6 mon					
	812-0910	812-0911	8AQ07C1	8AQ10C2	8AQ11C2
TSA Plates	$8.4x10^9$	$1.1x10^{10}$	$1.8x10^{10}$	$1.1x10^8$	$1.0x10^7$
MCA Plates	0	0	0	0	0
SDA Plates	$1.5x10^9$	$8.2x10^9$	$2.7x10^9$	$3.2x10^7$	$2.5x10^6$
BA Plates	$7.9x10^9$	$8.1x10^9$	$2.4x10^{10}$	$8.1x10^7$	$7.4x10^6$

Storage Stability of QST 713 Technical, Containing a Strain of *Bacillus subtilis*.: Lots Test Substance Titer [Colony forming Units (cfu/g)]: 12 mon					
	812-0910	812-0911	8AQ07C1	8AQ10C2	8AQ11C2
TSA Plates	$1.4x10^{10}$	$1.4x10^{10}$	$2.1x10^{10}$	$5.8x10^7$	$1.3x10^7$
MCA Plates	0	0	0	0	0
SDA Plates	$5.0x10^9$	$1.4x10^{10}$	$2.4x10^9$	$2.8x10^7$	$1.5x10^6$
BA Plates	$2.0x10^{10}$	$1.4x10^{10}$	$5.2x10^{10}$	$1.3x10^8$	$1.5x10^7$

II DISCUSSION

Colony forming units are evaluated on TSA plates. The overall counts of the five received lot production of MCPA is 1.3×10^7 to 2.1×10^{10} cfu/g (n=5). The longevity of QST 713 technical will be stable after 12 mon storage at ambient temperature, with a ± 30% change. The packet classification is SUPPLEMENTARY, and may be upgraded to ACCEPTABLE, with the submission of a clarification or justification of plating technique: "... allowed to stand before use" (7 of 18).

DATA EVALUATION REPORT

Reviewed by: Carl Etsitty, Microbiologist

Secondary Reviewer: John L. Kough, Ph.D., Senior Scientist

STUDY TYPE:	Manufacturing Process (OPPTS 885.1200)
MRID NO:	446519-04
TEST MATERIAL:	QST 713 Technical or Technical Powder (TP) (*Bacillus subtilis* Strain QST 713)
PROJECT NO:	None Assigned
SPONSOR:	AgraQuest, Inc., Davis, CA 95616
TESTING FACILITY:	AgraQuest, Inc., Davis, CA 95616
TITLE OF REPORT:	Manufacturing and Analytical Data for QST 713 Technical
AUTHOR(S):	Laura Cunningham Hilbig, and E.M. Bellet, Ph.D.
STUDY COMPLETED:	None Assigned
GOOD LABORATORY PRACTICE:	Non GLP Compliant
CONCLUSION:	General steps to cultivation/harvesting, using the cultured seed of *Bacillus subtilis* QST 713 as an inoculum: [redacted]
CLASSIFICATION:	SUPPLEMENTAL – May be upgraded to ACCEPTABLE with the following submissions: Proposed AI cfu range (upper/lower limit); Percent recovery error, based on sensitivity and limit of detection; Justification on pp 15 of 44, last paragraph, [redacted] Provide 5 dry and 2 additional broth production lot/batch; Justification of [redacted] imit of detection [redacted]

CONTAINS CONFIDENTIAL BUSINESS INFORMATION

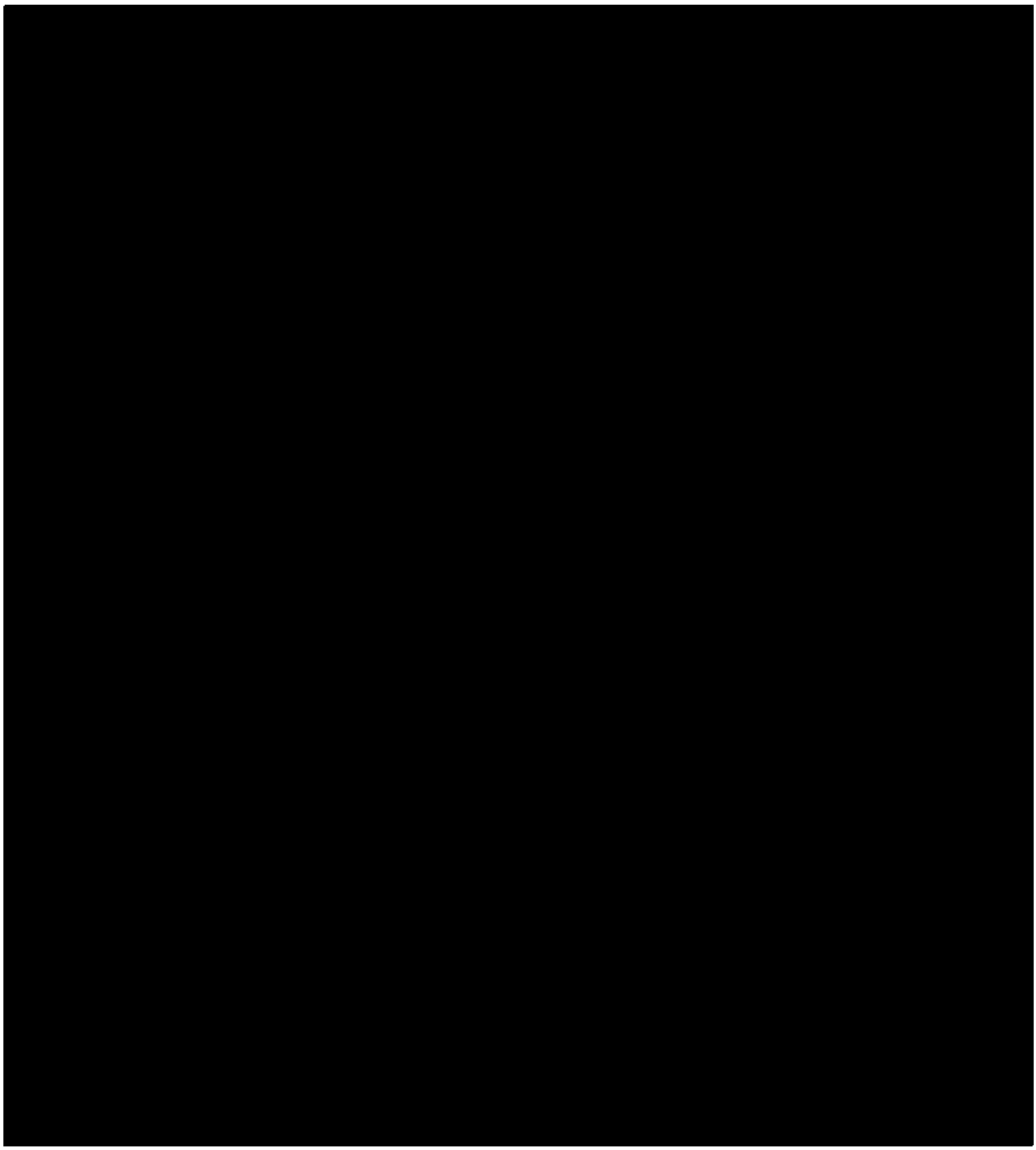
NOTE: After section 1.2.7 and 1.2.9, proceed onto appropriate End Use Manufacturing Process.

Manufacturing process information may be entitled to confidential treatment

1 **STUDY DESIGN**

1.1 Test Material: QST 713 Technical, active ingredient is *Bacillus subtilis* Strain QST 713

1.2 **MANUFACTURING PROCESS**:



Pages 66-69 - *Manufacturing process information may be entitled to confidential treatment*

2 **DISCUSSION**

[redacted]

3 **COMMENT**

[redacted]. Therefore, the packet is SUPPLEMENTAL, and may be up graded to ACCEPTABLE with the following submissions: Proposed AI cfu range (upper/lower limit); Percent recovery error, based on sensitivity and limit of detection; Justification on pp 15 of 44, last paragraph, [redacted] Provide 5 dry and 2 additional broth production lot/batch analysis; Justification of [redacted] limit of detection [redacted]

DATA EVALUATION REPORT

Reviewed by: Michael T. Watson, Ph.D., Plant Pathologist MTW

Secondary Reviewer: John L. Kough, Ph.D., Biologist JLK

STUDY TYPE:	Sensitivity of Detection
MRID NO:	446519-05
TEST MATERIAL:	QST 713 TP (Technical Powder)
PROJECT NO:	L08726 SN3
SPONSOR:	AgraQuest, Inc., Davis, Ca
TESTING FACILITY:	IIT Research Institute, Chicago, IL
TITLE OF REPORT:	Sensitivity of Detection of *Bacillus subtilis* Strain QST 713 for Toxicity/Pathogenicity Testing in Rats
AUTHOR(S):	Bruce A. Gingras, Ph.D.
STUDY COMPLETED:	August 20, 1998
CONCLUSION:	QST 713 technical powder was tested for the sensitivity of detection to support results of toxicity and pathogenicity testing in rats. Microbial recovery following addition of the test substance at a concentration of 4.3×10^{10} cfu/g, to lung and caecal tissue of male and female CD rats was determined. Test substance suspensions were mixed with tissue homogenates at levels of 10^2 and 10^4 cfu/ml and plated on trypticase soy agar (TSA) or TSA/Polymyxin B agar. Results indicate acceptable recovery for the lung tissue at both the 10^2 and 10^4 inoculum levels. For the caecal tissue, recovery was only acceptable for the 10^4 inoculum levels.
CLASSIFICATION:	ACCEPTABLE. This is not a guideline requirement, but is acceptable data to support the registration of the product.
GOOD LABORATORY PRACTICE:	This study was conducted in accordance with Good Laboratory Practice guidelines.

This study has been primarily reviewed by Richard A. Duncan of the California EPA. His review (Cal EPA Document # 52540-012; EPA Reg. # 69592; ID # 173883N; Record # 163739) is attached and is acceptable for the purposes of USEPA review for health effects.

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

TOXICOLOGY SUMMARY REPORT WORKSHEET

I. STUDY IDENTIFICATION

Active Ingredient: *Bacillus subtilis*, Strain QST 713
Formulated Product Name: QST 713 Technical
Chemical Code #: 5447 **ID #:** 173883N
Document #: 52540-012 **Record #:** 163739
EPA Reg. #: 69592- **SB 950 #:** New a.i.
Study Type: Sensitivity of Detection (no study type number)
Full Study Title: Sensitivity of Detection *of Bacillus subtilis* Strain QST 713 for Toxicity/Pathogenicity Testing in Rats
Company Sponsor: AgraQuest, Inc.
Conducting Laboratory: IIT Research Institute, Chicago, IL
Final Report Date: 8/98 **Laboratory Study #:** L08726SN3

CONCLUSIONS: Does this study indicate a possible adverse health effect? No

ONE LINER - Summary of the study:
012; 163739; "Sensitivity of Detection *of Bacillus subtilis* Strain QST 713 for Toxicity/-Pathogenicity Testing in Rats" (B. A. Gingras; IIT Research Institute, Chicago, IL; Lab Study No. L08726SN3; 8/98); the homogeneity and 3-h stability of aqueous suspensions of QST 713 Technical (Lot No. 8AQ07C2; 4.3 x 10^{10} CFU/g) and recovery from rat lung and cecum were evaluated; test article suspensions were mixed with tissue homogenates at levels of 10^2 and 10^4 CFU/ml either by vortexing or by blending, and then plated on either trypticase soy agar (TSA) or TSA/Polymyxin B (to reduce interference by competing bacteria); samples were plated in duplicate or triplicate and counted after incubation at 35°C for approx. 23 h; results indicated that TSA media are acceptable for enumeration of the test article from "sterile" tissues and TSA/P media are acceptable for "non-sterile" tissues; blending had no effect on test article recovery; suspensions of the test article were homogeneous and stable for 3 h; the test article could be accurately recovered from cecum samples only at the higher inoculum level (10^4 CFU/ml); Supplemental. (Duncan, 1/6/99)

Associate Pesticide Review Scientist	1-25-99 **Date**
Senior Toxicologist	1-27-99 **Date**

D52540>W163739.152

DATA EVALUATION REPORT

Reviewed by: Michael T. Watson, Ph.D., Plant Pathologist

Secondary Reviewer: John L. Kough, Ph.D., Biologist

STUDY TYPE:	Acute Oral Toxicity/Pathogenicity (152A-10)
MRID NO:	446519-06
TEST MATERIAL:	QST 713 Technical Powder (TP)
PROJECT NO:	L08726 SN4
SPONSOR:	AgraQuest, Inc., Davis, CA
TESTING FACILITY:	IIT Research Institute, Chicago, IL
TITLE OF REPORT:	Toxicity/Pathogenicity testing of QST 713 following acute oral challenge in rats
AUTHOR(S):	Kelly A. Harrington, B.S.
STUDY COMPLETED:	August, 1998 (signed by author, 8-24-98)
CONCLUSION:	QST 713 Technical was administered orally to male and female rats at a dose of approximately 1.13 x 10^8 cfu/animal in a 1 ml volume. The test microbe was recovered on Days 0-7 from the stomach/small intestines, caecum, and feces of test animals dosed with the technical powder, but the organism was cleared before the Day 14 observation. In addition, the test microbe was recovered from the lungs, liver and mesenteric lymph nodes of one female rat (#302) on Day 0 and from the mesenteric lymph nodes of another female rat (#301), also on Day 0. No adverse clinical signs were observed, and gross necropsy did not reveal any abnormalities.
CLASSIFICATION:	ACCEPTABLE
GOOD LABORATORY PRACTICE:	This study was conducted in accordance with Good Laboratory Practice guidelines

This study has been primarily reviewed by Richard A. Duncan of the California EPA. His review (Cal EPA Document # 52540-013; EPA Reg. # 69592; ID # 173883N; Record # 163771) is attached and is acceptable for the purposes of USEPA review for health effects.

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

TOXICOLOGY STUDY EVALUATION WORKSHEET
(acute)

I. STUDY IDENTIFICATION

Active Ingredient: *Bacillus subtilis*, Strain QST 713
Formulated Product Name: QST 713 Technical
Chemical Code #: 5447 **ID #:** 173883N
Document #: 52540-013 **Record #:** 163771
EPA Reg. #: 69592- **SB 950 #:** New a.i.
Study Type: 152A10 - Acute Oral Toxicity/Pathogenicity
Full Study Title: Toxicity/Pathogenicity Testing of QST 713 Following Acute Oral Challenge in Rats
Company Sponsor: AgraQuest, Inc.
Conducting Laboratory: IIT Research Institute, Chicago, IL
Final Report Date: 8/98 **Laboratory Study #:** L08726 SN4

II. SUMMARY OF WORKSHEET

A. STUDY STATUS: Is report complete? Yes **Is study acceptable?** Yes
Meets EPA guidelines? Yes **Has useful data?** Yes
Minor variances from guidelines? **Insufficient data?**
Major variances from guidelines? **Non EPA validated study?**
Could be upgraded with additional information (see VI-A)?

B. CONCLUSIONS: Does this study indicate a possible adverse health effect? No

C. ONE LINER - Summary of the study:
**013; 160771; 152a-10; "Toxicity/Pathogenicity Testing of QST 713 Following Acute Oral Challenge in Rats" (K. A. Harrington; IIT Research Institute, Chicago, IL; Lab Project No. L08726 SN4; 8/98); QST 713 Technical (Lot No. 8AQ07C2; 4 x 10^{10} CFU/g), dosed as a suspension in purified water; 0 (shelf controls) (3M/3F), 0 (isolated controls) (15M/15F), 0 (killed test substance) (15M/15F), 1 x 10^{8} CFU/animal (15M/15F); no mortality; Clinical Observations- no adverse signs observed; Infectivity/persistence- the test microbe was detected in the stomach/small intestines, cecum, feces, lungs, liver, and mesenteric lymph nodes of females and/or males dosed with the viable test article and was completely cleared by Day 14; the test microbe was not detected in animals of any other test group; Necropsy- no gross lesions; Organ Weights- no significant differences; no adverse effects; Acceptable. (Duncan, 1/14/98)

D. ARE DATA ADEQUATE TO SUPPORT REGISTRATION (if applicable)? Yes

Wayman — 1-25-99
Associate Pesticide Review Scientist **Date**

Peter Leung — 1-27-99
Senior Toxicologist **Date**

III. PROTOCOL SUMMARY

A. ANIMALS, ROUTE OF ADMINISTRATION, AND DURATION OF TREATMENT:

Species: Rat
Strain: Crl:CD
Source of animals: Charles River Breeding Laboratories, Raleigh, NC
Age at start: approx. 56 d
Route of administration: Oral gavage
Vehicle: Purified water
Duration of treatment: Single dose
Study dates: 4/13/98 - 5/6/98

B. BACKGROUND (including relationship of this study to other studies):

A preliminary lethality test was conducted by dosing three rats/sex with 9 x 10^7 CFU of the test article and observing them for four days. No signs of toxicity were observed.

C. TREATMENT LEVELS AND GROUP SIZE

Group	Treatment	Dose (CFU)	Number Treated Male	Female
1	Shelf Control [a] (untreated)	0	3	3
2	Naive (Isolated) Control [b] (untreated)	0	15	15
3	Killed Test Substance [b] (autoclaved QST 713 Technical/ H2O)	0 (1 x 10^8, killed)	15	15
4	Test Substance [b] (QST 713 Technical/H2O)	1 x 10^8	15	15

(a) Terminated on Day 14.
(b) Three animals/sex were killed on Days 0, 3, 7, 14. Three animals/sex were originally allocated for termination on Day 21, but these animals were not needed because complete clearance of the test article was observed by Day 14.

IV. STUDY DESIGN AND CONDUCT EVALUATION

A. STUDY PROCEDURES AND REMARKS (e.g., OK, specific parameters; asterisks denote deficiencies, NA indicates not applicable or no comment).

1. **Test article (assay, purity, lot #, stability):** QST 713 Technical (Lot No. 8AQ07C2; 4 x 10^{10} CFU/g), a light brown powder; dosed as a suspension in purified water
2. **Analysis of dosing material (stability, homogeneity, compound content):**OK, test article suspensions were found to be homogeneous, free of bacterial and fungal contamination, and stable for at least 2.75 h; dosing suspensions contained approx. 80-120% of target concentrations (pre-blended, TSA media)
3. **Animal selection (species, strain, age, sex):** OK
4. **Animal husbandry (housing, etc):** OK

5. **Mortality (and intercurrent disease):** None
6. **Number of animals (start and termination):** OK
7. **Randomization of animals:** OK
8. **Dose level selection (number of groups and justification):** OK
9. **Route of administration (appropriate for test article):** OK
10. **Exposure conditions (schedule and methods):** OK
11. **Controls (negative and positive):** OK, groups of untreated, untreated/isolated, and animals treated with killed test substance were included
12. **Observations (cageside, body weight, physicals, etc):** OK, animals were observed daily through Day 21 (termination); animals were weighed on Days 0, 3, 7, and 14
13. **Necropsies (required animals, tissues, or parameters):** OK, animals were killed by CO_2 inhalation and observed for abnormalities; blood, lungs, spleen, kidneys, liver, mesenteric lymph nodes, brain, stomach/small intestine, cecum, and feces were weighed and analyzed for presence of the test article using a microbial enumeration method that was validated in a previous study (see Record No. 163739; results repeated in Table 2); enumeration was conducted both before and after heat treatment to inactivate vegetative spores
14. **Appropriateness of methods:** OK
15. **Treatment of results (data summarization and statistics):** OK
16. **Study report (complete, reflects data, data cited but missing):** OK
17. **Consistency (with other studies of this type):** OK
18. **Good laboratory practice (internal audits, sign-offs):** OK
19. **Other:** NA

B. ELABORATION OF METHODS OR PROTOCOL DESCRIPTION (if needed): NA

V. RESULTS

A. EFFECTS REPORTED:

No mortality;

Clinical Observations- no adverse signs observed;

Infectivity/persistence- the test microbe was detected in the stomach/small intestines, cecum, and feces of males and females dosed with the viable test article, and in the lungs, liver, and mesenteric lymph nodes of females in the same group; the test microbe was cleared from all tissues by Day 14; the test microbe was not detected in animals of any other test group;

Necropsy- no gross lesions.

Organ Weights- there were no significant differences in organ weights.

B. ACUTE TOXICITY VALUE (LD50, LC50, etc.): NA

C. TOXICITY CATEGORY: NA

VI. DISCUSSION

A. MAJOR DEFICIENCIES (if present). What are they and can they be corrected with additional information? Be specific: NONE

B. DISCUSSION OF RESULTS (if necessary). Were there significant adverse health effects? NO **Are there any recommendations specific to this study?:** NONE

DATA EVALUATION REPORT

Reviewed by: Michael T. Watson, Ph.D., Plant Pathologist MTW

Secondary Reviewer: John L. Kough, Ph.D., Biologist JLK

STUDY TYPE:	Acute Dermal Toxicity/Pathogenicity (152A-11)
MRID NO:	446519-07
TEST MATERIAL:	QST 713 Technical Powder (TP)
PROJECT NO:	L08726 SN7
SPONSOR:	AgraQuest, Inc., Davis, CA
TESTING FACILITY:	IIT Research Institute, Chicago, IL
TITLE OF REPORT:	Acute Dermal Toxicity/Pathogenicity Study of QST 713 in Rabbits
AUTHOR(S):	John Findlay, B.S.
STUDY COMPLETED:	August 6, 1998
CONCLUSION:	Approximately 10% of the dorsal area fur of five male and five female rabbits was clipped and a dose of 2g/kg bodyweight was applied to the test site. The test site was covered with a 12.8 x 11.5 cm surgical dressing for 24 hours. Following the 24 hour exposure period, the residual test substance was removed with water moistened gauze pads. The animals were observed for clinical signs and/or skin irritation, "frequently" immediately following dosing and once per day for 13 days after removal of the wrapping. Edema, erythema and eschar formation was observed in all 10 rabbits, and multiple sores were observed in nine of the rabbits, following unwrapping. Necrosis was observed in some rabbits on Day 2, and in all rabbits by Day 4. Except for superficial skin flaking, all skin irritation effects were cleared in nine of the ten rabbits by Day 14. Rabbit #946 continued to exhibit edema, erythema, and eschar through Day 14. No rabbits died as a result of exposure to 2g/kg bodyweight of the test substance, therefore the LD_{50} of the test substance is greater than 2g/kg bodyweight.
CLASSIFICATION:	ACCEPTABLE, TOXICITY CATEGORY III
GOOD LABORATORY PRACTICE:	This study was conducted in accordance with Good Laboratory Practice guidelines

This study has been primarily reviewed by Richard A. Duncan of the California EPA. His review (Cal EPA Document # 52540-014; EPA Reg. # 69592; ID # 173883N; Record # 163772) is attached and is acceptable for the purposes of USEPA review for health effects.

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

TOXICOLOGY STUDY EVALUATION WORKSHEET
(acute)

I. STUDY IDENTIFICATION

Active Ingredient: *Bacillus subtilis*, Strain QST 713
Formulated Product Name: QST 713 Technical
Chemical Code #: 5447 **ID #:** 173883N
Document #: 52540-014 **Record #:** 163772
EPA Reg. #: 69592- **SB 950 #:** New a.i.
Study Type: 885.3100 - Acute Dermal Toxicity/Pathology
Full Study Title: Acute Dermal Toxicity/Pathology Study of QST 713 in Rabbits
Company Sponsor: AgraQuest, Inc.
Conducting Laboratory: IIT Research Institute, Chicago, IL
Final Report Date: 8/6/98 (amended) **Laboratory Study #:** L08726SN7

II. SUMMARY OF WORKSHEET

A. **STUDY STATUS: Is report complete?** Yes **Is study acceptable?** Yes
Meets EPA guidelines? Yes **Has useful data?** Yes
Minor variances from guidelines? **Insufficient data?**
Major variances from guidelines? **Non EPA validated study?**
Could be upgraded with additional information (see VI-A)?

B. **CONCLUSIONS: Does this study indicate a possible adverse health effect?** No

C. **ONE LINER - Summary of the study:**
**014; 163772; 885.3100; "Acute Dermal Toxicity/Pathology Study of QST 713 in Rabbits" (J. Findlay; IIT Research Institute, Chicago, IL; Lab Study No. L08726SN7; 8/6/98); QST 713 Technical (Lot No. 8AQ07C2; 4.7 x 10^{10} CFU/g), applied as an aqueous paste; 2 g/kg; 5 animals/sex; occlusive wrap, 24-hour exposure; no mortality; Clinical Observations- erythema, edema, necrosis, eschar, sores, cracking, flaking, and new or repaired skin were observed at the application site; LD50 (M and F) > 2 g/kg; Toxicity Category III; Acceptable. (Duncan, 1/6/99)

D. **ARE DATA ADEQUATE TO SUPPORT REGISTRATION (if applicable)?** Yes

Associate Pesticide Review Scientist — Date: 1-25-99

Senior Toxicologist — Date: 1-25-99

III. PROTOCOL SUMMARY

A. ANIMALS, ROUTE OF ADMINISTRATION, AND DURATION OF TREATMENT:

Species: Rabbit
Strain: New Zealand White
Source of animals: Kuiper Rabbit Ranch, Gary, IN
Age at start: approx. 3 mos.
Route of administration: Dermal, semi-occlusive wrap
Vehicle: None (prepared as a paste with 3 ml purified water/dose)
Duration of treatment: Single dose, 24-hour exposure period
Study dates: 4/15/98 - 4/29/98

B. BACKGROUND (including relationship of this study to other studies): NA

C. TREATMENT LEVELS AND GROUP SIZE

Group	Treatment	Dose (g/kg)	No. Dead/No. Dosed Male	Female
1	QST 713 Technical	2 [a]	0/5	0/5

(a) 2.30×10^{11} to 2.73×10^{11} CFU/animal.

IV. STUDY DESIGN AND CONDUCT EVALUATION

A. STUDY PROCEDURES AND REMARKS (e.g., OK, specific parameters; asterisks denote deficiencies, NA indicates not applicable or no comment).

1. **Test article (assay, purity, lot #, stability):** QST 713 Technical (Lot No. 8AQ07C2; 4.7×10^{10} CFU/g), a light brown powder; applied as a paste made by mixing each dose with 3 ml purified water
2. **Analysis of dosing material (stability, homogeneity, compound content):** OK
3. **Animal selection (species, strain, age, sex):** OK
4. **Animal husbandry (housing, etc):** OK
5. **Mortality (and intercurrent disease):** None
6. **Number of animals (start and termination):** OK
7. **Randomization of animals:** Not reported
8. **Dose level selection (number of groups and justification):** OK
9. **Route of administration (appropriate for test article):** OK
10. **Exposure conditions (schedule and methods):** OK
11. **Controls (negative and positive):** Not required
12. **Observations (cageside, body weight, physicals, etc):** OK, animals were observed frequently on day 1 (dosing) and once or twice daily through Day 14 (termination); dermal irritation was graded daily on days 2-14; week 0, 1, and 2 body weights were reported
13. **Necropsies (required animals, tissues, or parameters):** Animals were discarded without necropsy
14. **Appropriateness of methods:** OK
15. **Treatment of results (data summarization and statistics):** OK
16. **Study report (complete, reflects data, data cited but missing):** OK
17. **Consistency (with other studies of this type):** OK

18. **Good laboratory practice (internal audits, sign-offs):** OK
19. **Other:** NA

B. ELABORATION OF METHODS OR PROTOCOL DESCRIPTION (if needed): NA

V. RESULTS

A. EFFECTS REPORTED:
No mortality; Clinical Observations- erythema, edema, necrosis, eschar, sores, cracking, flaking, and new or repaired skin were observed at the application site

B. ACUTE TOXICITY VALUE (LD50, LC50, etc.): LD50 (M and F) > 2 g/kg

C. TOXICITY CATEGORY: III

VI. DISCUSSION

A. MAJOR DEFICIENCIES (if present). What are they and can they be corrected with additional information? Be specific: NONE

B. DISCUSSION OF RESULTS (if necessary). Were there significant adverse health effects? NO **Are there any recommendations specific to this study?:** NONE

DATA EVALUATION REPORT

Reviewed by: Michael T. Watson, Ph.D., Plant Pathologist MTW

Secondary Reviewer: John L. Kough, Ph.D., Biologist

STUDY TYPE:	Acute Intravenous Toxicity/Pathogenicity
MRID NO:	446519-08
TEST MATERIAL:	QST 713 Technical Powder (TP)
PROJECT NO:	L08726 SN5
SPONSOR:	AgraQuest, Inc., Davis, CA
TESTING FACILITY:	IIT Research Institute, Chicago, IL
TITLE OF REPORT:	Toxicity/Pathogenicity Testing of QST 713 following Acute Intravenous Challenge in Rats
AUTHOR(S):	Kelly A. Harrington, B.S.
STUDY COMPLETED:	August 1998 (signed by author, 8-21-98)
CONCLUSION:	A 1 g aliquot of the test substance (QST 713 TP) was diluted into 10 ml of water. The test substance was further diluted (1:215) to yield a concentration of 9.4×10^6 cfu in a volume of 0.5 ml for intravenous administration into rats. Control treatments included naive controls, shelf control and killed test substance groups. Rats, separated into appropriate groups, were sacrificed at 0, 7, 21, and 35 days post-dosing. There was no mortality and no adverse clinical signs were observed in any of the rats dosed with the test substance. The test microbe was cleared from most organs by the Day 35 observation. However, low levels of the organism continued to be detected in the spleen and the liver after 35 days. The microbe was not detected in any of the animals in the control groups.
CLASSIFICATION:	ACCEPTABLE
GOOD LABORATORY PRACTICE:	This study was conducted in accordance with Good Laboratory Practice guidelines

This study has been primarily reviewed by Richard A. Duncan of the California EPA. His review (Cal EPA Document # 52540-015; EPA Reg. # 69592; ID # 173883N; Record # 163773) is attached and is acceptable for the purposes of USEPA review for health effects.

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

TOXICOLOGY STUDY EVALUATION WORKSHEET
(acute)

I. STUDY IDENTIFICATION

Active Ingredient: *Bacillus subtilis*, Strain QST 713
Formulated Product Name: QST 713 Technical
Chemical Code #: 5447 **ID #:** 173883N
Document #: 52540-015 **Record #:** 163773
EPA Reg. #: 69592- **SB 950 #:** New a.i.
Study Type: 152A13 - Acute Intravenous Toxicity/Pathogenicity
Full Study Title: Toxicity/Pathogenicity Testing of QST 713 Following Acute Intravenous Challenge in Rats
Company Sponsor: AgraQuest, Inc.
Conducting Laboratory: IIT Research Institute, Chicago, IL
Final Report Date: 8/98 **Laboratory Study #:** L08726 SN5

II. SUMMARY OF WORKSHEET

A. STUDY STATUS: Is report complete? Yes **Is study acceptable?** Yes
Meets EPA guidelines? Yes **Has useful data?** Yes
Minor variances from guidelines? **Insufficient data?**
Major variances from guidelines? **Non EPA validated study?**
Could be upgraded with additional information (see VI-A)?

B. CONCLUSIONS: Does this study indicate a possible adverse health effect? No

C. ONE LINER - Summary of the study:
**015; 160773; 152a-13; "Toxicity/Pathogenicity Testing of QST 713 Following Acute Intravenous Challenge in Rats" (K. A. Harrington; IIT Research Institute, Chicago, IL; Lab Project No. L08726 SN5; 8/98); QST 713 Technical (Lot No. 8AQ07C2; 4.3 x 10^{10} CFU/g), dosed as a suspension in purified water; 0 (shelf controls) (3M/3F), 0 (isolated controls) (12M/12F), 0 (killed test substance) (12M/12F), 9.4 x 10^{6} CFU/animal (12M/12F); no mortality; Clinical Observations- no adverse signs observed; Infectivity/persistence- the test microbe was detected in the blood, lungs, spleen, kidneys, and liver of males and females dosed with the viable test article; the test microbe was cleared from most organs and tissues by Day 35, but remained at low levels in the spleen and liver; the test microbe was not detected in animals of any other test group; Necropsy- no gross lesions; Organ Weights- no significant differences; no adverse effects; Acceptable. (Duncan, 1/14/98)

D. ARE DATA ADEQUATE TO SUPPORT REGISTRATION (if applicable)? Yes

V. Duncan — 1-25-99
Associate Pesticide Review Scientist **Date**

Peter Lewis — 1-25-99
Senior Toxicologist **Date**

III. PROTOCOL SUMMARY

A. ANIMALS, ROUTE OF ADMINISTRATION, AND DURATION OF TREATMENT:

Species: Rat
Strain: Crl:CD
Source of animals: Charles River Breeding Laboratories, Raleigh, NC
Age at start: approx. 56 d
Route of administration: Intravenous injection
Vehicle: Purified water
Duration of treatment: Single dose
Study dates: 4/22/98 - 5/27/98

B. BACKGROUND (including relationship of this study to other studies):

A preliminary lethality test was conducted by dosing three rats/sex with 9 x 10^6 CFU of the test article and observing them for five days. Rough hair coat was observed in one male.

C. TREATMENT LEVELS AND GROUP SIZE

Group	Treatment	Dose (CFU)	Number Treated Male	Female
1	Shelf Control [a] (untreated)	0	3	3
2	Naive (Isolated) Control [b] (untreated)	0	12	12
3	Killed Test Substance [b] (autoclaved QST 713 Technical/ H2O)	0 (9.4 x 10^6, killed)	12	12
4	Test Substance [b] (QST 713 Technical/H2O)	9.4 x 10^6	12	12

(a) Terminated on Day 35.
(b) Three animals/sex were killed on Days 0, 7, 21, and 35.

IV. STUDY DESIGN AND CONDUCT EVALUATION

A. STUDY PROCEDURES AND REMARKS (e.g., OK, specific parameters; asterisks denote deficiencies, NA indicates not applicable or no comment).

1. **Test article (assay, purity, lot #, stability):** QST 713 Technical (Lot No. 8AQ07C2; 4.3 x 10^{10} CFU/g), a light brown powder; dosed as a suspension in purified water
2. **Analysis of dosing material (stability, homogeneity, compound content):** OK, test article suspensions were found to be homogeneous, free of bacterial and fungal contamination, and stable for at least 3 h; dosing suspensions contained approx. 80-120% of target concentrations (pre-blended, TSA media)
3. **Animal selection (species, strain, age, sex):** OK
4. **Animal husbandry (housing, etc):** OK
5. **Mortality (and intercurrent disease):** None
6. **Number of animals (start and termination):** OK

7. **Randomization of animals:** OK
8. **Dose level selection (number of groups and justification):** OK
9. **Route of administration (appropriate for test article):** OK
10. **Exposure conditions (schedule and methods):** OK
11. **Controls (negative and positive):** OK, groups of untreated, untreated/isolated, and animals treated with killed test substance were included
12. **Observations (cageside, body weight, physicals, etc):** OK, animals were observed twice daily, except weekends and holidays, through Day 35 (termination); animals were weighed on Days 0, 7, 14, 21, 28, and 35
13. **Necropsies (required animals, tissues, or parameters):** OK, animals were killed by CO_2 inhalation and observed for abnormalities; lungs, spleen, kidneys, liver, mesenteric lymph nodes, brain, and cecum were weighed and analyzed for presence of the test article using a microbial enumeration method that was validated in a previous study (see Record No. 163739; results repeated in Table 2); enumeration was conducted both before and after heat treatment to inactivate vegetative spores; blood was also analyzed for presence of the test article, but was not weighed
14. **Appropriateness of methods:** OK
15. **Treatment of results (data summarization and statistics):** OK
16. **Study report (complete, reflects data, data cited but missing):** OK
17. **Consistency (with other studies of this type):** OK
18. **Good laboratory practice (internal audits, sign-offs):** OK
19. **Other:** NA

B. ELABORATION OF METHODS OR PROTOCOL DESCRIPTION (if needed): NA

V. RESULTS

A. EFFECTS REPORTED:

No mortality;

Clinical Observations- no adverse signs observed;

Infectivity/persistence- the test microbe was detected in the blood, lungs, spleen, kidneys, and liver of males and females dosed with the viable test article; the test microbe was cleared from most organs and tissues by Day 35, but remained at low levels in the spleen and liver; the test microbe was not detected in animals of any other test group;

Organ Weights- there were no significant differences in organ weights;

Necropsy- no gross lesions.

B. ACUTE TOXICITY VALUE (LD50, LC50, etc.): NA

C. TOXICITY CATEGORY: NA

VI. DISCUSSION

A. MAJOR DEFICIENCIES (if present). What are they and can they be corrected with additional information? Be specific: NONE

B. DISCUSSION OF RESULTS (if necessary). Were there significant adverse health effects? NO **Are there any recommendations specific to this study?:** NONE

DATA EVALUATION REPORT

Reviewed by: Michael T. Watson, Ph.D., Plant Pathologist MTW

Secondary Reviewer: John L. Kough, Ph.D., Biologist JLK

STUDY TYPE:	Acute Pulmonary Toxicity/Pathogenicity (152A-12)
MRID NO:	446519-09
TEST MATERIAL:	QST 713 Technical Powder (TP)
PROJECT NO:	L08726 SN6
SPONSOR:	AgraQuest, Inc., Davis, CA
TESTING FACILITY:	IIT Research Institute, Chicago, IL
TITLE OF REPORT:	Toxicity/Pathogenicity Testing of QST 713 Following Acute Intratracheal Challenge in Rats
AUTHOR(S):	Kelly Harrington, B.S.
STUDY COMPLETED:	August, 1998 (signed by author, 8-21-98)
CONCLUSION:	Male and female rats were dosed intratracheally with QST 713 technical powder as a suspension in purified water at a concentration of 1.2×10^8 cfu/animal. Shelf control, killed test substance (KTG), and naive control (NC) rats groups were used as controls. Rats dosed with the test substance, as well as the KTG and NC rats were separated into groups (five rats/group/sex/day) for sacrifice at 0, 7, 21 and 35 days post dosing. There was no mortality as a result of the dosing, and other than a mottle lung parenchyma on Day 0, no other adverse effects were observed via gross necropsy. One male rat exhibited rough hair coat, but no other clinical signs were observed. Three female rats exhibited slight weight loss (one for two consecutive weeks), but the overall weight gain in all rats was similar. The test microbe was detectable (both pre- and post-heat treatments) in the lungs of the rats through Day 35, but at significantly reduced levels compared to Day 0. The organism was also detectable, post heat treatment, in the spleen, liver and kidneys of some of the animals through the Day 7 sacrifice. The organism was cleared in those organs in all animals by the Day 21 sacrifice.
CLASSIFICATION:	ACCEPTABLE
GOOD LABORATORY PRACTICE:	This study was conducted in accordance with Good Laboratory Practice guidelines

This study has been primarily reviewed by Richard A. Duncan of the California EPA. His review (Cal EPA Document # 52540-016; EPA Reg. # 69592; ID # 173883N; Record # 163774) is attached and is acceptable for the purposes of USEPA review for health effects.

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

TOXICOLOGY STUDY EVALUATION WORKSHEET
(acute)

I. STUDY IDENTIFICATION

Active Ingredient: *Bacillus subtilis*, Strain QST 713
Formulated Product Name: QST 713 Technical
Chemical Code #: 5447 **ID #:** 173883N
Document #: 52540-016 **Record #:** 163774
EPA Reg. #: 69592- **SB 950 #:** New a.i.
Study Type: 152A12 - Acute Pulmonary Toxicity/Pathogenicity
Full Study Title: Toxicity/Pathogenicity Testing of QST 713 Following Acute Intratracheal Challenge in Rats
Company Sponsor: AgraQuest, Inc.
Conducting Laboratory: IIT Research Institute, Chicago, IL
Final Report Date: 8/98 **Laboratory Study #:** L08726 SN6

II. SUMMARY OF WORKSHEET

A. STUDY STATUS: Is report complete? Yes **Is study acceptable?** Yes
Meets EPA guidelines? Yes **Has useful data?** Yes
Minor variances from guidelines? **Insufficient data?**
Major variances from guidelines? **Non EPA validated study?**
Could be upgraded with additional information (see VI-A)?

B. CONCLUSIONS: Does this study indicate a possible adverse health effect? No

C. ONE LINER - Summary of the study:
**016; 160774; 152a-12; "Toxicity/Pathogenicity Testing of QST 713 Following Acute Intratracheal Challenge in Rats" (K. A. Harrington; IIT Research Institute, Chicago, IL; Lab Project No. L08726 SN6; 8/98); QST 713 Technical (Lot No. 8AQ07C2; 4.3 x 10^{10} CFU/g), dosed as a suspension in purified water; 0 (shelf controls) (5M/5F), 0 (isolated controls) (20M/20F), 0 (killed test substance) (20M/20F), 1.2 x 10^{8} CFU/animal (20M/20F); no mortality; Clinical Observations- rough hair coat in one male; reduced weight gain during week 1 (M and F) and 3 (F only), but no differences in total (Day 0-35) weight gain; Infectivity/persistence- test microbe detected (pre- and post heat treatment) in the lungs through Day 35, estimated clearance by Day 92-108; also detected, post-heat treatment, in spleen, liver, and kidneys, but it was cleared by Day 21; Organ Weights- relative lung weight was increased on Days 0 (M and F) and 7 (M only); Necropsy- mottled lung parenchyma on Day 0; no adverse effects; Acceptable. (Duncan, 1/22/98)

D. ARE DATA ADEQUATE TO SUPPORT REGISTRATION (if applicable)? Yes

Associate Pesticide Review Scientist — Date: 1-25-99

Senior Toxicologist — Date: 1-27-99

III. PROTOCOL SUMMARY

A. ANIMALS, ROUTE OF ADMINISTRATION, AND DURATION OF TREATMENT:

Species: Rat
Strain: Crl:CD
Source of animals: Charles River Laboratories, Raleigh, NC
Age at start: approx. 58 d
Route of administration: Intratracheal instillation
Vehicle: Purified water
Duration of treatment: Single dose
Study dates: 4/23/98 - 5/28/98

B. BACKGROUND (including relationship of this study to other studies):

A preliminary lethality test was conducted by dosing three rats/sex with 9.05 x 10^7 CFU of the test article and observing them for five days. No signs of toxicity were observed.

C. TREATMENT LEVELS AND GROUP SIZE

Group	Treatment	Dose (CFU)	Number Treated Male	Female
1	Shelf Control[a] (untreated)	0	5	5
2	Naive (Isolated) Control[b] (untreated)	0	20	20
3	Killed Test Substance[b] (autoclaved QST 713 Technical/ H2O)	0 (1.2 x 10^8, killed)	20	20
4	Test Substance[b] (QST 713 Technical/H2O)	1.2 x 10^8	20	20

(a) Terminated on Day 35.
(b) Five animals/sex were killed on Days 0, 7, 21, and 35.

IV. STUDY DESIGN AND CONDUCT EVALUATION

A. STUDY PROCEDURES AND REMARKS (e.g., OK, specific parameters; asterisks denote deficiencies, NA indicates not applicable or no comment).

1. **Test article (assay, purity, lot #, stability):** QST 713 Technical (Lot No. 8AQ07C2; 4.3 x 10^{10} CFU/g), a light brown powder; dosed as a suspension in purified water
2. **Analysis of dosing material (stability, homogeneity, compound content):** OK, test article suspensions were found to be homogeneous, free of bacterial and fungal contamination, and stable for at least 3 h; dosing suspensions contained approx. 80-120% of target concentrations (pre-blended, TSA media)
3. **Animal selection (species, strain, age, sex):** OK
4. **Animal husbandry (housing, etc):** OK
5. **Mortality (and intercurrent disease):** None
6. **Number of animals (start and termination):** OK

7. **Randomization of animals:** OK
8. **Dose level selection (number of groups and justification):** OK
9. **Route of administration (appropriate for test article):** OK
10. **Exposure conditions (schedule and methods):** OK
11. **Controls (negative and positive):** OK, groups of untreated, untreated/isolated, and animals treated with killed test substance were included
12. **Observations (cageside, body weight, physicals, etc):** OK, animals were observed twice daily, except weekends and holidays, through Day 35 (termination); animals were weighed on Days 0, 7, 14, 21, 28, and 35
13. **Necropsies (required animals, tissues, or parameters):** OK, animals were killed by CO_2 asphyxiation and observed for abnormalities; lungs with associated lymph nodes, spleen, kidneys, liver, brain, and cecum were weighed and analyzed for presence of the test article using a microbial enumeration method that was validated in a previous study (see Record No. 163739); enumeration was conducted both before and after heat treatment to inactivate vegetative spores; blood was also analyzed for presence of the test article, but was not weighed
14. **Appropriateness of methods:** OK
15. **Treatment of results (data summarization and statistics):** OK
16. **Study report (complete, reflects data, data cited but missing):** OK
17. **Consistency (with other studies of this type):** OK
18. **Good laboratory practice (internal audits, sign-offs):** OK
19. **Other:** NA

B. ELABORATION OF METHODS OR PROTOCOL DESCRIPTION (if needed):

V. RESULTS

A. EFFECTS REPORTED:

No mortality;

Clinical Observations- rough hair coat was observed in one male in the test substance group; reduced weight gain was observed in males and females in the test substance group during the first week, and for females during the third week; increased weight gain was observed in males during the fourth week; there were no significant differences in total (Day 0-35) weight gain for either sex;

Infectivity/persistence- the test microbe was detected (pre- and post-heat treatment) in the lungs and associated lymph nodes in males and females dosed with the test substance at all necropsy intervals; the data showed that the microbe was slowly being cleared from the lungs and was estimated to be completely cleared in 92-108 days; in post-heat treatment samples, the test microbe was also detected in the spleen, liver, and kidneys, but it was cleared by Day 21; the test microbe was not detected in animals of any other test group;

Organ Weights- relative lung weight was significantly increased in the test substance group on Days 0 (both sexes) and 7 (males only); other relative organ weights were significantly reduced: liver in test substance males on Day 7, spleen in killed test substance males on Day 7, and spleen in test substance and killed test substance females on Day 0;

Necropsy- mottled lung parenchyma on Day 0 in all animals dosed with the test substance.

B. ACUTE TOXICITY VALUE (LD50, LC50, etc.): NA

C. TOXICITY CATEGORY: NA

VI. DISCUSSION

A. MAJOR DEFICIENCIES (if present). What are they and can they be corrected with additional information? Be specific: NONE

B. DISCUSSION OF RESULTS (if necessary). Were there significant adverse health effects? NO **Are there any recommendations specific to this study?:** NONE

DATA EVALUATION REPORT

Reviewed by: Michael T. Watson, Ph.D., Plant Pathologist MTW

Secondary Reviewer: John L. Kough, Ph.D., Biologist JLK

STUDY TYPE:	Acute Inhalation
MRID NO:	446527-05
TEST MATERIAL:	QST 713 Wettable Powder (WP)
PROJECT NO:	3474.1
SPONSOR:	AgraQuest, Inc., Davis, CA
TESTING FACILITY:	Springborn Laboratories, Inc., Spencerville, OH
TITLE OF REPORT:	An Acute Whole-Body Inhalation Toxicity Study in Rats with QST 713 WP
AUTHOR(S):	George A. Douds, M.S.
STUDY COMPLETED:	August 28, 1998
CONCLUSION:	The four hour whole-body inhalation toxicity of QST 713 WP was evaluated in 10 Sprague-Dawley rats (5 male and 5 female). The rats received a time-weighted average aerosol concentration of 0.63 mg/L (the maximum maintainable concentration which gave a median aerodynamic particle size less than 4.0μm). Some clinical signs and weight loss were noted, but no mortality resulted from the dosing. A gross necropsy at the end of the 14 day observation period did not reveal any abnormalities. However, the test was performed with material diluted in water at a 25% w/w concentration, and therefore, an actual concentration was not determined. Despite this shortcoming, the aerodynamic particle size data for the dry aerosol indicate that approximately 64% of the particles are larger than 8.8μm in diameter and approximately 94% of the particles are larger than 5.1μm in diameter, with a Mass Median Aerodynamic Diameter (MMAD) of 10.5μm (GSD = 1.6). Normally, particles of this size pose a low risk of inhalation exposure. Therefore, although this report is classified as SUPPLEMENTAL, it will not affect the classification of the submission, based upon the low risk of exposure to the product.
CLASSIFICATION:	SUPPLEMENTAL. Can be upgraded to ACCEPTABLE with submission of analytical data which indicates the actual concentration used in the dosing.
GOOD LABORATORY PRACTICE:	This study was conducted in accordance with Good Laboratory Practice Standards

This study has been primarily reviewed by Richard A. Duncan of the California EPA. His review (Cal EPA Document# 52540-008; EPA Reg. # 69592; ID# 173883N; Record# 163771) is attached. The study is considered to be supplementary, however, revision is not necessary as this data would not significantly affect an EPA decision on the acceptability of the product.

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

TOXICOLOGY STUDY EVALUATION WORKSHEET
(acute)

I. STUDY IDENTIFICATION

Active Ingredient: *Bacillus subtilis*, Strain QST 713
Formulated Product Name: QST 713 WP
Chemical Code #: 5447 **ID #:** 173883N
Document #: 52540-008 **Record #:** 163735
EPA Reg. #: 69592- **SB 950 #:** New a.i.
Study Type: 813 - Acute Inhalation
Full Study Title: An Acute Whole-Body Inhalation Toxicity Study in Rats with QST 713 WP
Company Sponsor: AgraQuest, Inc.
Conducting Laboratory: Springborn Laboratories, Inc., Spencerville, OH
Final Report Date: 8/28/98 **Laboratory Study #:** 3474.1

II. SUMMARY OF WORKSHEET

A. **STUDY STATUS: Is report complete?** No **Is study acceptable?** No
Meets EPA guidelines? **Has useful data?** Yes
Minor variances from guidelines? **Insufficient data?**
Major variances from guidelines? Yes **Non EPA validated study?**
Could be upgraded with additional information (see VI-A)? Yes

B. **CONCLUSIONS: Does this study indicate a possible adverse health effect?** No

C. **ONE LINER - Summary of the study:**
008; 163735; 813; "An Acute Whole-Body Inhalation Toxicity Study in Rats with QST 713" (G. A. Douds; Springborn Laboratories, Inc., Spencerville, OH; Lab Study No.3474.1; 8/28/98); QST 713 WP (Lot No. 32.38.10B), prepared as a 25% w/w mixture in deionized water before generation as a liquid aerosol; 0.63 mg/l (gravimetric); 5 animals/sex; 4-hour, whole-body exposure; MMAD = 3.8 um (GSD = 1.9); no mortality; Clinical Observations- included salivation, breathing abnormalities, decreased activity, wobbly gait, apparent hypothermia, hunched posture, corneal opacity, decreased defecation, urine stain, decreased food consumption, dark material around facial area, weight loss; Necropsy- no significant internal findings; LC50 and Toxicity Category not determined; Unacceptable but may be upgraded by submitting analytical exposure concentration data. (Duncan, 1/4/99)

D. **ARE DATA ADEQUATE TO SUPPORT REGISTRATION (if applicable)?** Yes

V Duncan 1-25-99
Associate Pesticide Review Scientist **Date**

Peter Leung 1-27-99
Senior Toxicologist **Date**

III. PROTOCOL SUMMARY

A. ANIMALS, ROUTE OF ADMINISTRATION, AND DURATION OF TREATMENT:

Species: Rat
Strain: Sprague Dawley
Source of animals: Harlan Sprague Dawley, Inc., Madison, WI, and Prattville, AL
Age at start: Not reported (young adults)
Route of administration: Liquid aerosol inhalation, whole-body exposure
Vehicle: Deionized water
Duration of treatment: Single, 4-hour exposure
Study dates: 8/3/98 - 8/17/98

B. BACKGROUND (including relationship of this study to other studies):

Numerous aerosol generation trials were conducted to determine the optimum equipment and conditions for use in the definitive study (details in Appendix A, p. 29) with the goal of achieving the highest attainable concentration with an MMAD of less than 4.0 um. Diluted and undiluted test article was used in the trials. In one trial with the undiluted test article, the maximum concentration generated was 2.24 mg/l, but the MMAD was 10.5 um (GSD = 1.6).

C. TREATMENT LEVELS AND GROUP SIZE

Group	Treatment	Exposure Conc.[a] (mg/l)	No. Dead/No. Dosed Male	Female
1	QST 713 WP/water	0.63	0/5	0/5

(a) Gravimetric concentration.

IV. STUDY DESIGN AND CONDUCT EVALUATION

A. STUDY PROCEDURES AND REMARKS (e.g., OK, specific parameters; asterisks denote deficiencies, NA indicates not applicable or no comment).

1. **Test article (assay, purity, lot #, stability):** QST 713 WP (Lot No. 32.38.10B), a light brown powder; the exposure atmosphere was generated from a 25% w/w mixture of the test article with deionized water
* 2. **Analysis of dosing material (stability, homogeneity, compound content):** Not reported
3. **Animal selection (species, strain, age, sex):** OK
4. **Animal husbandry (housing, etc):** OK
5. **Mortality (and intercurrent disease):** None
6. **Number of animals (start and termination):** OK
7. **Randomization of animals:** Selected arbitrarily
8. **Dose level selection (number of groups and justification):** OK, the exposure concentration generated was considered to be "the maximum that could be maintained and that would give a median aerodynamic particle size less than 4.0 um."
9. **Route of administration (appropriate for test article):** OK
** 10. **Exposure conditions (schedule and methods):** Gravimetric samples were collected approximately every 30 mins. (10 samples total) on glass fiber filters. The time-weighted average concentration was reported. The mean and standard deviation of the ten samples was 0.62 mg/l (0.06). Analytical concentration data were not reported.

Two cascade impactor samples were collected and the data were combined to calculate particle size. The MMAD was 3.8 um (GSD = 1.9).

11. **Controls (negative and positive):** Not required
12. **Observations (cageside, body weight, physicals, etc):** OK, animals were observed twice on Day 0 (exposure) and then daily through Day 14 (termination); animals were weighed on Days 0, 7, and 14
13. **Necropsies (required animals, tissues, or parameters):** OK
14. **Appropriateness of methods:** OK
15. **Treatment of results (data summarization and statistics):** OK
16. **Study report (complete, reflects data, data cited but missing):** OK
17. **Consistency (with other studies of this type):** OK
18. **Good laboratory practice (internal audits, sign-offs):** OK
19. **Other:** NA

B. ELABORATION OF METHODS OR PROTOCOL DESCRIPTION (if needed):

V. RESULTS

A. EFFECTS REPORTED:
No mortality; Clinical Observations- included salivation, breathing abnormalities, decreased activity, wobbly gait, apparent hypothermia, hunched posture, corneal opacity, decreased defecation, urine stain, decreased food consumption, dark material around facial area, weight loss; Necropsy- no significant internal findings

B. ACUTE TOXICITY VALUE (LD_{50}, LC_{50}, etc.): Not determined

C. TOXICITY CATEGORY: Not determined

VI. DISCUSSION

A. MAJOR DEFICIENCIES (if present). What are they and can they be corrected with additional information? Be specific:
Current guidelines require reporting the actual concentration of the test article in the breathing zone. Since the test article was diluted in a vehicle before generation of the exposure atmosphere, chemical analysis of samples is required to determine the concentration of test article (separate from the vehicle). However, only gravimetric data was submitted.

This deficiency may be corrected by submitting analytical exposure concentration data.

B. DISCUSSION OF RESULTS (if necessary). Were there significant adverse health effects? NO **Are there any recommendations specific to this study?:**
Aerodynamic particle size data for a dry aerosol of the undiluted test article (p. 35) show that approx. 64% of the particles are larger than 8.8 um in diameter and approx. 94% are larger than 5.1 um in diameter. The MMAD was 10.5 um (GSD = 1.6). Particles of this size pose a low risk of inhalation exposure.

DATA EVALUATION REPORT

Reviewed by: Michael T. Watson, Ph.D., Plant Pathologist MTW

Secondary Reviewer: John L. Kough, Ph.D., Biologist JLK

STUDY TYPE:	Primary Dermal Irritation
MRID NO:	446646-01
TEST MATERIAL:	QST 713 TP
PROJECT NO:	0420XA54.004
SPONSOR:	AgraQuest, Inc., Davis, CA
TESTING FACILITY:	Chrysalis Preclinical Services, Olyphant, PA
TITLE OF REPORT:	Primary Dermal Irritation in Rabbits with QST 713 TP
AUTHOR(S):	Victor T. Mallory, B.S., RLAT
STUDY COMPLETED:	August 6, 1998
CONCLUSION:	Approximately 500 mg of the test substance was applied to one intact site on the clipped dorsal trunk of each of six New Zealand White rabbits (3 male and 3 female). The test substance was moistened with 0.3 ml of saline, and covered with a gauze patch for a four hour exposure period. After 4 hours, the gauze was removed, and the residual test substance was removed using gauze moistened with water. The animals were examined for clinical and toxicological signs at 30 & 60 minutes, and 24, 48 and 72 hours (± 1 hour) after gauze removal. One animal lost a very small amount of weight (initial = 3.457 kg; final = 3.418 kg). Four animals showed slight erythema at the 30-60 minute evaluation, and three of these animals continued to show very slight erythema at the 24 hour observation. All of the these signs were resolved by the 48 hour observation.
CLASSIFICATION:	ACCEPTABLE. TOXICITY CATEGORY IV
GOOD LABORATORY PRACTICE:	This study was conducted in accordance with Good Laboratory Practice guidelines

This study has been primarily reviewed by Richard A. Duncan of the California EPA. His review (Cal EPA Document # 52540-017; EPA Reg. # 69592; ID # 173883N; Record # 163775) is attached and is acceptable for the purposes of USEPA review for health effects.

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

TOXICOLOGY STUDY EVALUATION WORKSHEET
(acute)

I. STUDY IDENTIFICATION

Active Ingredient: *Bacillus subtilis*, Strain QST 713
Formulated Product Name: QST 713 TP
Chemical Code #: 5447 **ID #:** 173883N
Document #: 52540-017 **Record #:** 163775
EPA Reg. #: 69592- **SB 950 #:** New a.i.
Study Type: 815 - Primary Dermal Irritation
Full Study Title: Primary Dermal Irritation in Rabbits with QST 713 TP
Company Sponsor: AgraQuest, Inc.
Conducting Laboratory: Chrysalis, Olyphant, PA
Final Report Date: 8/20/98 (amended) **Laboratory Study #:** 0420XA54.004

II. SUMMARY OF WORKSHEET

A. **STUDY STATUS: Is report complete?** Yes **Is study acceptable?** Yes
Meets EPA guidelines? Yes **Has useful data?** Yes
Minor variances from guidelines? **Insufficient data?**
Major variances from guidelines? **Non EPA validated study?**
Could be upgraded with additional information (see VI-A)?

B. **CONCLUSIONS: Does this study indicate a possible adverse health effect?** No

C. **ONE LINER - Summary of the study:**
**017; 163775; 815; "Primary Dermal Irritation in Rabbits with QST 713 TP" (V. T. Mallory; Chrysalis, Olyphant, PA; Lab Study No. 0420XA54.004; 8/20/98 (amended)); QST 713 TP (Lot No. 8AQ07C2), moistened with distilled water (0.3 ml/dose); 500 mg/site; one intact site/animal, 6 animals; occlusive wrap, 4-hour exposure; examined 30-60 mins., and 24, 48, and 72 h (termination) after exposure; erythema of 0-1 at 30-60 mins. and 24 h, clear by 48 h; Toxicity Category IV; Acceptable. (Duncan, 1/5/99)

D. **ARE DATA ADEQUATE TO SUPPORT REGISTRATION (if applicable)?** Yes

V. Bynum 1-25-99
Associate Pesticide Review Scientist **Date**

Peter Leung 1-28-99
Senior Toxicologist **Date**

III. PROTOCOL SUMMARY

A. ANIMALS, ROUTE OF ADMINISTRATION, AND DURATION OF TREATMENT:

Species: Rabbit
Strain: New Zealand White
Source of animals: Hare-Marland
Age at start: approx. 18 wks
Route of administration: Dermal, one intact site, occlusive wrap
Vehicle: None (0.3 ml saline was used to moisten each dose)
Duration of treatment: Single dose; 4-hour exposure period
Study dates: 6/25/98 - 6/28/98

B. BACKGROUND (including relationship of this study to other studies): NA

C. TREATMENT LEVELS AND GROUP SIZE

Group	Treatment	Dose	Number of Animals
1	QST 713 TP	500 mg/site	6

IV. STUDY DESIGN AND CONDUCT EVALUATION

A. STUDY PROCEDURES AND REMARKS (e.g., OK, specific parameters; asterisks denote deficiencies, NA indicates not applicable or no comment).

1. **Test article (assay, purity, lot #, stability):** QST 713 TP (Lot No. 8AQ07C2), a light brown powder
2. * **Analysis of dosing material (stability, homogeneity, compound content):** Not reported
3. **Animal selection (species, strain, age, sex):** OK
4. **Animal husbandry (housing, etc):** OK
5. **Mortality (and intercurrent disease):** None
6. **Number of animals (start and termination):** OK
7. **Randomization of animals:** OK
8. **Dose level selection (number of groups and justification):** OK
9. **Route of administration (appropriate for test article):** OK
10. **Exposure conditions (schedule and methods):** OK
11. **Controls (negative and positive):** OK
12. **Observations (cageside, body weight, physicals, etc):** OK, skin was examined 30-60 mins., and 24, 48, and 72 h (termination) after exposure
13. **Necropsies (required animals, tissues, or parameters):** NA
14. **Appropriateness of methods:** OK
15. **Treatment of results (data summarization and statistics):** OK
16. **Study report (complete, reflects data, data cited but missing):** OK
17. **Consistency (with other studies of this type):** OK
18. **Good laboratory practice (internal audits, sign-offs):** OK
19. **Other:** NA

B. ELABORATION OF METHODS OR PROTOCOL DESCRIPTION (if needed): NA

V. RESULTS

A. EFFECTS REPORTED:
Range of scores for intact skin (number of animals with scores > 0):

	30-60 mins.	24 h	48 h	72 h (term.)
Erythema	0,1 (4/6)	0,1 (3/6)	Clear -->	
Edema	No edema --->			

B. ACUTE TOXICITY VALUE (LD_{50}, LC_{50}, etc.): NA

C. TOXICITY CATEGORY: IV

VI. DISCUSSION

A. MAJOR DEFICIENCIES (if present). What are they and can they be corrected with additional information? Be specific: NONE

B. DISCUSSION OF RESULTS (if necessary). Were there significant adverse health effects? NO **Are there any recommendations specific to this study?:** NONE

DATA EVALUATION REPORT

Reviewed by: Michael T. Watson, Ph.D., Plant Pathologist MTW

Secondary Reviewer: John L. Kough, Ph.D., Biologist JLK

STUDY TYPE:	Primary Eye Irritation (152A-14)
MRID NO:	446646-02
TEST MATERIAL:	QST 713 Technical Powder (TP)
PROJECT NO:	0421XA54.004
SPONSOR:	AgraQuest, Inc., Davis, CA
TESTING FACILITY:	Chrysalis Preclinical Services, Olyphant, PA
TITLE OF REPORT:	Primary Eye Irritation in Rabbits with QST 713 WP
AUTHOR(S):	Victor T. Mallory, B.S., RLAT
STUDY COMPLETED:	August 6, 1998
CONCLUSION:	A 0.1 ml (packed volume) sample of the test substance was placed into the conjunctival sac of the right eye of three male and three female New Zealand White rabbits. For each animal, the left eye of each served as an untreated control. The animals were examined at 1, 4, 24, 48, & 72 hours, as well as 4 days post- dosing and scored for ocular irritation. One animal (# 1883) lost a small amount of weight (304g) through the observation period. All six of the animals showed slight conjunctival effects through the 24 hour observation and three of the six animals continued to show slight conjunctival irritation through the 72 hour observation. In addition, 3/6, 2/6 and 1/6 of the animals showed slight iris effects through 24, 48 and 72 hours respectively. All of the signs cleared before the 96 hour observation.
CLASSIFICATION:	ACCEPTABLE. TOXICITY CATEGORY III
GOOD LABORATORY PRACTICE:	This study was conducted in accordance with Good Laboratory Practice guidelines

This study has been primarily reviewed by Richard A. Duncan of the California EPA. His review (Cal EPA Document # 52540-018; EPA Reg. # 69592; ID # 173883N; Record # 163776) is attached and is acceptable for the purposes of USEPA review for health effects.

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

TOXICOLOGY STUDY EVALUATION WORKSHEET
(acute)

I. STUDY IDENTIFICATION

Active Ingredient: *Bacillus subtilis*, Strain QST 713
Formulated Product Name: QST 713 TP
Chemical Code #: 5447 **ID #:** 173883N
Document #: 52540-018 **Record #:** 163776
EPA Reg. #: 69592- **SB 950 #:** New a.i.
Study Type: 814 - Primary Eye Irritation
Full Study Title: Primary Eye Irritation in Rabbits with QST 713 TP
Company Sponsor: AgraQuest, Inc.
Conducting Laboratory: Chrysalis, Olyphant, PA
Final Report Date: 8/20/98 (amended) **Laboratory Study #:** 0421XA54.004

II. SUMMARY OF WORKSHEET

A. STUDY STATUS: Is report complete? Yes **Is study acceptable?** Yes
Meets EPA guidelines? Yes **Has useful data?** Yes
Minor variances from guidelines? **Insufficient data?**
Major variances from guidelines? **Non EPA validated study?**
Could be upgraded with additional information (see VI-A)?

B. CONCLUSIONS: Does this study indicate a possible adverse health effect? No

C. ONE LINER - Summary of the study:
**018; 163776; 814; "Primary Eye Irritation in Rabbits with QST 713 TP" (V. T. Mallory; Chrysalis, Olyphant, PA; Lab Study No. 0421XA54.004; 8/20/98 (amended)); QST 713 TP (Lot No. 8AQ07C2), applied undiluted; 0.1 ml/eye; 6 animals unwashed; examined at 1, 24, 48, 72, and 96 h (termination); iritis (max. score = 1) and conjunctivitis (max. scores = 2/redn., 3/chem., 1/disch.); all effects cleared by 96 h; Toxicity Category III; Acceptable. (Duncan, 1/6/99)

D. ARE DATA ADEQUATE TO SUPPORT REGISTRATION (if applicable)? Yes

V Duncan
Associate Pesticide Review Scientist 1-25-99 **Date**

Peter Leung
Senior Toxicologist 1-28-99 **Date**

III. PROTOCOL SUMMARY

A. ANIMALS, ROUTE OF ADMINISTRATION, AND DURATION OF TREATMENT:

Species: Rabbit
Strain: New Zealand White
Source of animals: Hare-Marland
Age at start: approx. 18 wks
Route of administration: Topical, placed in conjunctival sac
Vehicle: None
Duration of treatment: Single dose
Study dates: 7/10/98 - 7/14/98

B. BACKGROUND (including relationship of this study to other studies): NA

C. TREATMENT LEVELS AND GROUP SIZE

Group	Treatment	Dose	Number of Animals
1	QST 713 TP, Unrinsed	0.1 ml [a]	6

(a) Dose weight was 81.0 - 99.9 mg.

IV. STUDY DESIGN AND CONDUCT EVALUATION

A. STUDY PROCEDURES AND REMARKS (e.g., OK, specific parameters; asterisks denote deficiencies, NA indicates not applicable or no comment).

1. **Test article (assay, purity, lot #, stability):** QST 713 TP (Lot No. 8AQ07C2), a light brown powder
2. **Analysis of dosing material (stability, homogeneity, compound content):** Not reported
3. **Animal selection (species, strain, age, sex):** OK
4. **Animal husbandry (housing, etc):** OK
5. **Mortality (and intercurrent disease):** None
6. **Number of animals (start and termination):** OK
7. **Randomization of animals:** Not reported
8. **Dose level selection (number of groups and justification):** OK
9. **Route of administration (appropriate for test article):** OK
10. **Exposure conditions (schedule and methods):** OK
11. **Controls (negative and positive):** OK
12. **Observations (cageside, body weight, physicals, etc):** OK, eyes were examined at 1, 24, 48, 72, and 96 h (termination); animals were weighed at the beginning and end of the study
13. **Necropsies (required animals, tissues, or parameters):** NA
14. **Appropriateness of methods:** OK
15. **Treatment of results (data summarization and statistics):** OK
16. **Study report (complete, reflects data, data cited but missing):** OK. The original report is dated 8/6/98. The report was amended on 8/20/98 to change the name of the test article from QST713WP to QST713TP.
17. **Consistency (with other studies of this type):** OK

18. **Good laboratory practice (internal audits, sign-offs):** OK
19. **Other:** NA

B. ELABORATION OF METHODS OR PROTOCOL DESCRIPTION (if needed): NA

V. RESULTS

A. EFFECTS REPORTED:
Range of scores for unwashed eyes (number of animals with scores > 0):

	1 h	24 h	48 h	72 h	96 h (term.)
Corneal Opacity	No opacity -->				
Iritis	0,1 (2/6)	0,1 (3/6)	0,1 (2/6)	0,1 (1/6)	Clear
Conjunctiva:					
Redness	2 (6/6)	1,2 (6/6)	0,1 (3/6)	0,1 (3/6)	Clear
Chemosis	1-3 (6/6)	0,1 (1/6)	Clear -->		
Discharge	1 (6/6)	Clear -->			

B. ACUTE TOXICITY VALUE (LD_{50}, LC_{50}, etc.): NA

C. TOXICITY CATEGORY: III

VI. DISCUSSION

A. MAJOR DEFICIENCIES (if present). What are they and can they be corrected with additional information? Be specific: NONE

B. DISCUSSION OF RESULTS (if necessary). Were there significant adverse health effects? NO **Are there any recommendations specific to this study?:** NONE

DATA EVALUATION REPORT

Reviewed by: Michael T. Watson, Ph.D., Plant Pathologist MTW

Secondary Reviewer: John L. Kough, Ph.D., Biologist JLK

STUDY TYPE:	Acute Oral Toxicity (152A-10)
MRID NO:	446647-01
TEST MATERIAL:	QST 713 WP (wettable powder)
PROJECT NO:	0402XA54.001
SPONSOR:	AgraQuest, Inc., Davis, CA
TESTING FACILITY:	Chrysalis Preclinical Services, Olyphant, PA
TITLE OF REPORT:	Acute Oral Exposure Toxicity Study in Rats with QST 713 WP
AUTHOR(S):	Victor T. Mallory, B.S., RLAT
STUDY COMPLETED:	August 5, 1998
CONCLUSION:	Five male and five female Sprague-Dawley Rats (Crl:CD®(SD)BR) were orally-dosed with 5000 mg/kg bodyweight of QST 173 wettable powder. Clinical observations were recorded at 1 and 4 hours post dosing, and daily through the 15 day observation period. None of the animals exhibited an abnormal clinical signs. Two female rats lost a small amount of weight (1 and 4 grams respectively) between the 8 and 15 day observation points, but both exhibited overall weight gain of 38 and 25 grams respectively. No abnormal effects were observed upon gross necropsy.
CLASSIFICATION:	ACCEPTABLE, Toxicity Category IV
GOOD LABORATORY PRACTICE:	This study was conducted in accordance with Good Laboratory Practice guidelines.

This study has been primarily reviewed by Richard A. Duncan of the California EPA. His review (Cal EPA Document# 52540-006; EPA Reg.# 69592; ID# 173883N; Record# 163733) is attached and is acceptable for the purposes of USEPA review for health effects.

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

TOXICOLOGY STUDY EVALUATION WORKSHEET
(acute and special studies)

I. STUDY IDENTIFICATION

Active Ingredient: *Bacillus subtilis*, Strain QST 713
Formulated Product Name: QST 713 WP
Chemical Code #: 5447 **ID #:** 173883N
Document #: 52540-006 **Record #:** 163733
EPA Reg. #: 69592- **SB 950 #:** New a.i.
Study Type: 811 - Acute Oral
Full Study Title: Acute Oral Exposure Toxicity in Rats with QST 713 WP
Company Sponsor: AgraQuest, Inc.
Conducting Laboratory: Chrysalis, Olyphant, PA
Final Report Date: 8/5/98 **Laboratory Study #:** 0402XA54.001

II. SUMMARY OF WORKSHEET

A. STUDY STATUS: Is report complete? Yes **Is study acceptable?** Yes
Meets EPA guidelines? Yes **Has useful data?** Yes
Minor variances from guidelines? **Insufficient data?**
Major variances from guidelines? **Non EPA validated study?**
Could be upgraded with additional information (see VI-A)?

B. CONCLUSIONS: Does this study indicate a possible adverse health effect? No

C. ONE LINER-One or two sentence summary of the study:
**006; 163733; 811; "Acute Oral Exposure Toxicity in Rats with QST 713 WP" (V. T. Mallory; Chrysalis, Olyphant, PA; Lab Study No. 0402XA54.001; 8/5/98); QST 713 WP (Lot No. 32.38.3B), dosed as a mixture in distilled water; 5000 mg/kg; 5 animals/sex; no mortality; Clinical Observations- normal; Necropsy- normal; LD50 (M and F) > 5000 mg/kg; Toxicity Category IV; Acceptable. (Duncan, 1/4/99)

D. ARE DATA ADEQUATE TO SUPPORT REGISTRATION (if applicable)?: Yes

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N Wyman 1-25-99
Associate Pesticide Review Scientist **Date**

Peter Leung 1-27-99
Senior Toxicologist **Date**

III. PROTOCOL SUMMARY

A. ANIMALS, ROUTE OF ADMINISTRATION, AND DURATION OF TREATMENT:

Species: Rat
Strain: Crl:CD(SD)BR
Source of animals: Charles River Laboratories, Inc.
Age at start: 8-9 wks
Route of administration: Oral gavage
Vehicle: Distilled water
Duration of treatment: Single dose
Study dates: 6/16/98 - 6/30/98

B. BACKGROUND (including relationship of this study to other studies): NA

C. TREATMENT LEVELS AND GROUP SIZE:

Group	Treatment	Dose (mg/kg)	No. Dead/No. Dosed Male	Female
1	QST 713 WP/dH_2O	5000	0/5	0/5

IV. STUDY DESIGN AND EVALUATION

A. TEST PROCEDURES AND REMARKS (e.g., OK, specific parameters; asterisks denote deficiencies, NA indicates not applicable or no comment).

1. **Test article (assay, purity, lot #, stability):** QST 713 WP (Lot No. 32.38.3B), a brown powder; dosed as a mixture in distilled water at a volume of 10 ml/kg
* 2. **Analysis of dosing material (stability, homogeneity, compound content):** Not reported
3. **Animal selection (species, strain, age, sex):** OK
4. **Animal husbandry (housing, etc):** OK
5. **Mortality (and intercurrent disease):** None
6. **Number of animals (start and termination):** OK
7. **Randomization of animals:** Not reported
8. **Dose level selection (number of groups and justification):** OK
9. **Route of administration (appropriate for test article):** OK
10. **Exposure conditions (schedule and methods):** OK
11. **Controls (negative and positive):** Not required
12. **Observations (cageside, body weight, physicals, etc):** OK, animals were observed twice on the day of dosing and then at least once daily through Day 15 (termination); animals were weighed on days 1, 8, and 15
13. **Necropsies (required animals, tissues, or parameters):** OK
14. **Appropriateness of methods:** OK
15. **Treatment of results (data summarization and statistics):** OK
16. **Study report (complete, reflects data, data cited but missing):** OK
17. **Consistency (with other studies of this type):** OK
18. **Good laboratory practice (internal audits, sign-offs):** OK
19. **Other:** NA

B. ELABORATION OF METHODS OR PROTOCOL DESCRIPTION (if needed): NA

V. RESULTS

A. EFFECTS REPORTED:
No mortality; Clinical Observations- normal; Necropsy- normal

B. ACUTE TOXICITY VALUE (LD50, LC50, etc.): LD50 (M and F) > 5000 mg/kg

C. TOXICITY CATEGORY: IV

VI. DISCUSSION

A. MAJOR DEFICIENCIES (if present). What are they and can they be corrected with additional information? Be specific: NONE

B. DISCUSSION OF RESULTS (if necessary). Were there significant adverse health effects?
NO **Are there any recommendations specific to this study?:** NONE

DATA EVALUATION REPORT

Reviewed by: Michael T. Watson, Ph.D., Plant Pathologist MTW

Secondary Reviewer: John L. Kough, Ph.D., Biologist JLK

STUDY TYPE:	Acute Dermal Toxicity (152A-11)
MRID NO:	446647-02
TEST MATERIAL:	QST 713 WP (wettable powder)
PROJECT NO:	0422XA54.001
SPONSOR:	AgraQuest, Inc., Davis, CA
TESTING FACILITY:	Chrysalis Preclinical Services, Olyphant, PA
TITLE OF REPORT:	Acute Exposure Dermal Toxicity in Rabbits with QST 713 WP
AUTHOR(S):	Victor T. Mallory, B.S., RLAT
STUDY COMPLETED:	August 5, 1998
CONCLUSION:	QST 713 wettable powder was applied to the prepared skin of 10 (five male and five female) New Zealand white rabbits at a concentration of 2000 mg/kg. Clinical observations were recorded at the time of unwrapping, and daily through the 15 day observation period. One of the animals (#1894) displayed an abnormal stance on days 3-6, but no other abnormal clinical signs were observed in any of the animals during the observation period. Also, there were signs of irritation and necrosis at the application site in some of the animals (individual animals were not identified). All of the animals gained weight throughout the study and a gross necropsy did not reveal any abnormal effects of the treatment.
CLASSIFICATION:	ACCEPTABLE, Toxicity Category III
GOOD LABORATORY PRACTICE:	This study was performed in accordance with Good Laboratory Practice guidelines.

This study has been primarily reviewed by Richard A. Duncan of the California EPA. His review (Cal EPA Document# 52540-007; EPA Reg.# 69592; ID# 173883N; Record# 163736) is attached and is acceptable for the purposes of USEPA review for health effects.

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

TOXICOLOGY STUDY EVALUATION WORKSHEET
(acute)

I. STUDY IDENTIFICATION

Active Ingredient: *Bacillus subtilis*, Strain QST 713
Formulated Product Name: QST 713 WP
Chemical Code #: 5447 **ID #:** 173883N
Document #: 52540-007 **Record #:** 163734
EPA Reg. #: 69592- **SB 950 #:** New a.i.
Study Type: 812 - Acute Dermal
Full Study Title: Acute Exposure Dermal Toxicity in Rabbits with QST 713 WP
Company Sponsor: AgraQuest, Inc.
Conducting Laboratory: Chrysalis, Olyphant, PA
Final Report Date: 8/5/98 **Laboratory Study #:** 0422XA54.001

II. SUMMARY OF WORKSHEET

A. **STUDY STATUS: Is report complete?** Yes **Is study acceptable?** Yes
Meets EPA guidelines? **Has useful data?** Yes
Minor variances from guidelines? Yes **Insufficient data?**
Major variances from guidelines? **Non EPA validated study?**
Could be upgraded with additional information (see VI-A)?

B. **CONCLUSIONS: Does this study indicate a possible adverse health effect?** No

C. **ONE LINER - Summary of the study:**
**007; 163734; 812; "Acute Exposure Dermal Toxicity in Rabbits with QST 713 WP" (V. T. Mallory; Chrysalis, Olyphant, PA; Lab Study No. 0422XA54.001; 8/5/98); QST 713 WP (Lot No. 32.38.3B), moistened with distilled water; 2000 mg/kg; 5 animals/sex; semi-occlusive wrap, 24-hour exposure; no mortality; Clinical Observations- abnormal stance; erythema, edema, necrosis, fissuring, and sloughing of skin at application site; Necropsy- no visible lesions; LD50 (M and F) > 2000 mg/kg; Toxicity Category III; Acceptable. (Duncan, 1/4/99)

D. **ARE DATA ADEQUATE TO SUPPORT REGISTRATION (if applicable)?**

M A Yamin — 1-25-99
Associate Pesticide Review Scientist **Date**

Peter Leung — 1-27-99
Senior Toxicologist **Date**

III. PROTOCOL SUMMARY

A. ANIMALS, ROUTE OF ADMINISTRATION, AND DURATION OF TREATMENT:

Species: Rabbit
Strain: New Zealand White
Source of animals: Hare-Marland
Age at start: 14 wks
Route of administration: Dermal, semi-occlusive wrap
Vehicle: None (1.5 ml distilled water was used to moisten each dose)
Duration of treatment: Single dose, 24-hour exposure period
Study dates: 6/17/98 - 7/1/98

B. BACKGROUND (including relationship of this study to other studies): NA

C. TREATMENT LEVELS AND GROUP SIZE

Group	Treatment	Dose (mg/kg)	No. Dead/No. Dosed Male	Female
1	QST 713 WP	2000	0/5	0/5

IV. STUDY DESIGN AND CONDUCT EVALUATION

A. STUDY PROCEDURES AND REMARKS (e.g., OK, specific parameters; asterisks denote deficiencies, NA indicates not applicable or no comment).

1. **Test article (assay, purity, lot #, stability):** QST 713 WP (Lot No. 32.38.3B), a light brown powder
* 2. **Analysis of dosing material (stability, homogeneity, compound content):** Not reported
3. **Animal selection (species, strain, age, sex):** OK
4. **Animal husbandry (housing, etc):** OK
5. **Mortality (and intercurrent disease):** None
6. **Number of animals (start and termination):** OK
7. **Randomization of animals:** Not reported
8. **Dose level selection (number of groups and justification):** OK
9. **Route of administration (appropriate for test article):** OK
* 10. **Exposure conditions (schedule and methods):** A minimal amount of fluid was used to moisten the test article
11. **Controls (negative and positive):** Not required
12. **Observations (cageside, body weight, physicals, etc):** OK, animals were observed at least once daily through Day 15 (termination); animals were weighed on days 1, 8, and 15
13. **Necropsies (required animals, tissues, or parameters):** OK
14. **Appropriateness of methods:** OK
15. **Treatment of results (data summarization and statistics):** OK
* 16. **Study report (complete, reflects data, data cited but missing):** The dosing and wrapping procedure was not described; paragraph three of the summary (p. 7) states that "no clinical or dermal signs were observed in the study", however this is inconsistent with statements in paragraph 5 and on p. 12, and data presented in the tables.

17. **Consistency (with other studies of this type):** OK
18. **Good laboratory practice (internal audits, sign-offs):** OK
19. **Other:** NA

B. ELABORATION OF METHODS OR PROTOCOL DESCRIPTION (if needed): NA

V. RESULTS

A. EFFECTS REPORTED:
No mortality; Clinical Observations- abnormal stance; erythema, edema, necrosis, fissuring, and sloughing of skin at application site; Necropsy- no visible lesions

B. ACUTE TOXICITY VALUE (LD50, LC50, etc.): LD50 (M and F) > 2000 mg/kg

C. TOXICITY CATEGORY: III

VI. DISCUSSION

A. MAJOR DEFICIENCIES (if present). What are they and can they be corrected with additional information? Be specific: NONE

B. DISCUSSION OF RESULTS (if necessary). Were there significant adverse health effects? NO **Are there any recommendations specific to this study?:** NONE

DATA EVALUATION REPORT

Reviewed by: Michael T. Watson, Ph.D., Plant Pathologist MTW

Secondary Reviewer: John L. Kough, Ph.D., Biologist JLK

STUDY TYPE:	Primary Dermal Irritation
MRID NO:	446647-03
TEST MATERIAL:	QST 713 Wettable Powder (WP)
PROJECT NO:	0420XA54.003
SPONSOR:	AgraQuest, Inc., Davis, CA
TESTING FACILITY:	Chrysalis Preclinical Services, Olyphant, PA
TITLE OF REPORT:	Primary Dermal Irritation in Rabbits with QST 713 WP
AUTHOR(S):	Victor T. Mallory, B.S., RLAT
STUDY COMPLETED:	August 5, 1998
CONCLUSION:	Five-hundred mg of QST 713 WP, moistened with 0.2 ml of saline, was applied to the clipped dorsal area of six New Zealand white rabbits. After a four hour exposure period, each rabbit was examined for signs of dermal irritation and scored according to Draize. All six animals showed very slight erythema at the 30-60 minute evaluations, with these signs resolving in each animal by the 72 hour observation. In addition, two animals showed very slight edema at the 30-60 minute (# 1888 & 1889) and at the 24 and 48 hour observations (#1886 & 1889). No other clinical signs were noted through 72 hours, and no animals exhibited weight loss as a result of the dosing.
CLASSIFICATION:	ACCEPTABLE
GOOD LABORATORY PRACTICE:	This study was conducted in accordance with Good Laboratory Practice guidelines.

This study has been primarily reviewed by Richard A. Duncan of the California EPA. His review (Cal EPA Document # 52540-009; EPA Reg. # 69592; ID # 173883N; Record # 163736) is attached and is acceptable for the purposes of USEPA review for health effects.

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

TOXICOLOGY STUDY EVALUATION WORKSHEET
(acute)

I. STUDY IDENTIFICATION

Active Ingredient: *Bacillus subtilis*, Strain QST 713
Formulated Product Name: QST 713 WP
Chemical Code #: 5447 **ID #:** 173883N
Document #: 52540-009 **Record #:** 163736
EPA Reg. #: 69592- **SB 950 #:** New a.i.
Study Type: 815 - Primary Dermal Irritation
Full Study Title: Primary Dermal Irritation in Rabbits with QST 713 WP
Company Sponsor: AgraQuest, Inc.
Conducting Laboratory: Chrysalis, Olyphant, PA
Final Report Date: 8/5/98 **Laboratory Study #:** 0420XA54.003

II. SUMMARY OF WORKSHEET

A. STUDY STATUS: Is report complete? Yes **Is study acceptable?** Yes
Meets EPA guidelines? Yes **Has useful data?** Yes
Minor variances from guidelines? **Insufficient data?**
Major variances from guidelines? **Non EPA validated study?**
Could be upgraded with additional information (see VI-A)?

B. CONCLUSIONS: Does this study indicate a possible adverse health effect? No

C. ONE LINER - Summary of the study:
**009; 163736; 815; "Primary Dermal Irritation in Rabbits with QST 713 WP" (V. T. Mallory; Chrysalis, Olyphant, PA; Lab Study No. 0420XA54.003; 8/5/98); QST 713 WP (Lot No. 32.38.3B), moistened with distilled water; 500 mg/site; one intact site/animal, 6 animals; semi-occlusive wrap, 4-hour exposure; examined 30-60 mins., and 24, 48, and 72 h (termination) after exposure; erythema of 1 and edema of 0-1 at 30-60 mins., erythema and edema of 0-1 at 24 h, erythema of 0-1 at 48 h; clear by 72 h; Toxicity Category IV; Acceptable. (Duncan, 1/5/99)

D. ARE DATA ADEQUATE TO SUPPORT REGISTRATION (if applicable)? Yes

[signature] 1-25-99
Associate Pesticide Review Scientist **Date**

Peter Lewis 1-27-99
Senior Toxicologist **Date**

III. PROTOCOL SUMMARY

A. ANIMALS, ROUTE OF ADMINISTRATION, AND DURATION OF TREATMENT:

Species: Rabbit
Strain: New Zealand White
Source of animals: Hare-Marland
Age at start: approx. 18 wks
Route of administration: Dermal, one intact site, occlusive wrap
Vehicle: None (0.2 ml saline was used to moisten each dose)
Duration of treatment: Single dose; 4-hour exposure period
Study dates: 6/16/98 - 6/19/98

B. BACKGROUND (including relationship of this study to other studies): NA

C. TREATMENT LEVELS AND GROUP SIZE

Group	Treatment	Dose	Number of Animals
1	QST 713 WP	500 mg/site	6

IV. STUDY DESIGN AND CONDUCT EVALUATION

A. STUDY PROCEDURES AND REMARKS (e.g., OK, specific parameters; asterisks denote deficiencies, NA indicates not applicable or no comment).

1. **Test article (assay, purity, lot #, stability):** QST 713 WP (Lot No. 32.38.3B), a light brown powder
2. * **Analysis of dosing material (stability, homogeneity, compound content):** Not reported
3. **Animal selection (species, strain, age, sex):** OK
4. **Animal husbandry (housing, etc):** OK
5. **Mortality (and intercurrent disease):** None
6. **Number of animals (start and termination):** OK
7. **Randomization of animals:** OK
8. **Dose level selection (number of groups and justification):** OK
9. **Route of administration (appropriate for test article):** OK
10. **Exposure conditions (schedule and methods):** OK
11. **Controls (negative and positive):** OK
12. **Observations (cageside, body weight, physicals, etc):** OK, skin was examined 30-60 mins., and 24, 48, and 72 h (termination) after exposure
13. **Necropsies (required animals, tissues, or parameters):** NA
14. **Appropriateness of methods:** OK
15. **Treatment of results (data summarization and statistics):** OK
16. **Study report (complete, reflects data, data cited but missing):** OK
17. **Consistency (with other studies of this type):** OK
18. **Good laboratory practice (internal audits, sign-offs):** OK
19. **Other:** NA

B. ELABORATION OF METHODS OR PROTOCOL DESCRIPTION (if needed): NA

V. RESULTS

A. EFFECTS REPORTED:

Range of scores for intact skin (number of animals with scores > 0):

	30-60 mins.	24 h	48 h	72 h (term.)
Erythema	1 (6/6)	0,1 (4/6)	0,1 (2/6)	Clear
Edema	0,1 (2/6)	0,1 (2/6)	Clear --->	

B. ACUTE TOXICITY VALUE (LD_{50}, LC_{50}, etc.): NA

C. TOXICITY CATEGORY: IV

VI. DISCUSSION

A. MAJOR DEFICIENCIES (if present). What are they and can they be corrected with additional information? Be specific: NONE

B. DISCUSSION OF RESULTS (if necessary). Were there significant adverse health effects? NO **Are there any recommendations specific to this study?:** NONE

DATA EVALUATION REPORT

Reviewed by: Michael T. Watson, Ph.D., Plant Pathologist MTW

Secondary Reviewer: John L. Kough, Ph.D., Biologist JLK

STUDY TYPE:	Primary Eye Irritation (152A-14)
MRID NO:	446647-04
TEST MATERIAL:	QST 713 Wettable Powder (WP)
PROJECT NO:	0421XA54.003
SPONSOR:	AgraQuest, Inc., Davis, CA
TESTING FACILITY:	Chrysalis Preclinical Services, Olyphant, PA
TITLE OF REPORT:	Primary Eye Irritation in Rabbits with QST 713 WP
AUTHOR(S):	Victor T. Mallory, B.S., RLAT
STUDY COMPLETED:	August 5, 1998
CONCLUSION:	Approximately 100 mg (0.1 ml packed volume) of QST 713 WP was placed into the right eye of six (three male and three female) New Zealand white rabbits. The eyes of each animal were examined at 1, 24, 48, and 72 hours post dosing. All animals exhibited slight to moderate irritation of the conjunctivae (redness, chemosis and/or discharge) at the 1 hour observation. Slight redness persisted in four animals at the 24 hour observation and in three animals at the 48 hour observation. All of the irritation signs were resolved before the 72 hour observation.
CLASSIFICATION:	ACCEPTABLE, Toxicity Category IV
GOOD LABORATORY PRACTICE:	This study was performed in accordance with Good Laboratory Practice guidelines.

This study has been primarily reviewed by Richard A. Duncan of the California EPA. His review (Cal EPA Document #52540-010; EPA Reg. #69592; ID # 173883N; Record #163737) is attached and is acceptable for the purposes of the USEPA review for health effects.

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

TOXICOLOGY STUDY EVALUATION WORKSHEET
(acute)

I. STUDY IDENTIFICATION

Active Ingredient: *Bacillus subtilis*, Strain QST 713
Formulated Product Name: QST 713 WP
Chemical Code #: 5447 **ID #:** 173883N
Document #: 52540-010 **Record #:** 163737
EPA Reg. #: 69592- **SB 950 #:** New a.i.
Study Type: 814 - Primary Eye Irritation
Full Study Title: Primary Eye Irritation in Rabbits with QST 713 WP
Company Sponsor: AgraQuest, Inc.
Conducting Laboratory: Chrysalis, Olyphant, PA
Final Report Date: 8/5/98 **Laboratory Study #:** 0421XA54.003

II. SUMMARY OF WORKSHEET

A. **STUDY STATUS: Is report complete?** Yes **Is study acceptable?** Yes
Meets EPA guidelines? Yes **Has useful data?** Yes
Minor variances from guidelines? **Insufficient data?**
Major variances from guidelines? **Non EPA validated study?**
Could be upgraded with additional information (see VI-A)?

B. **CONCLUSIONS: Does this study indicate a possible adverse health effect?** No

C. **ONE LINER - Summary of the study:**
**010; 163737; 814; "Primary Eye Irritation in Rabbits with QST 713 WP" (V. T. Mallory; Chrysalis, Olyphant, PA; Lab Study No. 0421XA54.003; 8/5/98); QST 713 WP (Lot No. 32.38.3B), applied undiluted; 0.1 ml/eye; 6 animals unwashed; examined at 1, 24, 48, and 72 h (termination); conjunctivitis only (max. scores = 2/redn., 1/chem., 1/disch.); all positive effects cleared by 24 h; Toxicity Category IV; Acceptable. (Duncan, 1/5/99)

D. **ARE DATA ADEQUATE TO SUPPORT REGISTRATION (if applicable)?** Yes

Associate Pesticide Review Scientist — Date: 1-25-99

Senior Toxicologist — Date: 1-27-99

III. PROTOCOL SUMMARY

A. ANIMALS, ROUTE OF ADMINISTRATION, AND DURATION OF TREATMENT:

Species: Rabbit
Strain: New Zealand White
Source of animals: Hare-Marland
Age at start: approx. 20 wks
Route of administration: Topical, placed in conjunctival sac
Vehicle: None
Duration of treatment: Single dose
Study dates: 6/26/98 - 6/29/98

B. BACKGROUND (including relationship of this study to other studies): NA

C. TREATMENT LEVELS AND GROUP SIZE

Group	Treatment	Dose	Number of Animals
1	QST 713 WP, Unrinsed	0.1 ml	6

IV. STUDY DESIGN AND CONDUCT EVALUATION

A. STUDY PROCEDURES AND REMARKS (e.g., OK, specific parameters; asterisks denote deficiencies, NA indicates not applicable or no comment).

1. **Test article (assay, purity, lot #, stability):** QST 713 WP (Lot No. 32.38.3B), a light brown powder
* 2. **Analysis of dosing material (stability, homogeneity, compound content):** Not reported
3. **Animal selection (species, strain, age, sex):** OK
4. **Animal husbandry (housing, etc):** OK
5. **Mortality (and intercurrent disease):** None
6. **Number of animals (start and termination):** OK
7. **Randomization of animals:** Not reported
8. **Dose level selection (number of groups and justification):** OK
9. **Route of administration (appropriate for test article):** OK
10. **Exposure conditions (schedule and methods):** OK
11. **Controls (negative and positive):** OK
12. **Observations (cageside, body weight, physicals, etc):** OK, eyes were examined at 1, 24, 48, and 72 h (termination)
13. **Necropsies (required animals, tissues, or parameters):** NA
14. **Appropriateness of methods:** OK
15. **Treatment of results (data summarization and statistics):** OK
16. **Study report (complete, reflects data, data cited but missing):** OK
17. **Consistency (with other studies of this type):** OK
18. **Good laboratory practice (internal audits, sign-offs):** OK
19. **Other:** NA

B. ELABORATION OF METHODS OR PROTOCOL DESCRIPTION (if needed): NA

V. RESULTS

A. EFFECTS REPORTED:
Range of scores for unwashed eyes (number of animals with scores > 0):

	1 h	24 h	48 h	72 h (term.)
Corneal Opacity	No opacity -->			
Iritis	No iritis -->			
Conjunctiva				
Redness	1,2 (6/6)	0,1 (4/6)	0,1 (3/6)	Clear
Chemosis	0,1 (2/6)	Clear -->		
Discharge	0,1 (1/6)	Clear -->		

B. ACUTE TOXICITY VALUE (LD_{50}, LC_{50}, etc.): NA

C. TOXICITY CATEGORY: IV

VI. DISCUSSION

A. MAJOR DEFICIENCIES (if present). What are they and can they be corrected with additional information? Be specific: NONE

B. DISCUSSION OF RESULTS (if necessary). Were there significant adverse health effects? NO **Are there any recommendations specific to this study?:** NONE

DATA EVALUATION REPORT

Reviewed by: Michael T. Watson, Ph.D., Plant Pathologist MTW

Secondary Reviewer: John L. Kough, Ph.D., Biologist JLK

STUDY TYPE:	Delayed Contact Hypersensitivity
MRID NO:	446647-05
TEST MATERIAL:	QST 713 Wettable Powder (WP)
PROJECT NO:	0424XA54.001
SPONSOR:	AgraQuest, Inc., Davis, CA
TESTING FACILITY:	Chrysalis Preclinical Services, Olyphant, PA
TITLE OF REPORT:	Delayed Contact Hypersensitivity in Guinea Pigs with QST 713 WP
AUTHOR(S):	Victor T. Mallory, B.S., RLAT
STUDY COMPLETED:	8-21-98
CONCLUSION:	Induction with the test material, QST 713 WP, did elicit a very mild delayed contact hypersensitivity response in guinea pigs which were challenged and rechallenged with the test material.
CLASSIFICATION:	ACCEPTABLE. The product should be labeled as a potential dermal sensitizer.
GOOD LABORATORY PRACTICE:	This study was conducted in accordance with Good Laboratory Practice guidelines

I. STUDY DESIGN

Test Material: The test material was QST 713 WP (Lot # 32.32.3B), a light brown powder which contains *Bacillus subtilis* strain QST 713 as the active ingredient.

Test Animals: Eighteen male and 18 female Hartley guinea pigs [Elm:(HA) - Elm Hill Breeding Laboratories] of approximately 5 weeks of age, were used in the study. The animals weighed between 324 and 448 grams at the beginning of the study.

Methods: *Treatment Groups:*

Group/Treatment	Males	Females
Vehicle Control	381-385	386-390
Test Article	5681-5690	5691-5700

Positive Control	391-393	394-396

Site Preparation/Treatment
Sites of application were clipped free of hair the day prior to treatment. The test material was applied to a 25 mm Hilltop Chamber® patch (the test substance was applied neat and moistened with 0.3 ml distilled water). The test substance was allowed skin contact for approximately 6 hours, at which time the patch was removed.

Dose-Range Finding Study:

Prior to experimental initiation, the irritation potential of the test substance was determined. Four animals was each treated at four sites with the test article at 10, 25, 50 and 100% strength for a six hour exposure period. All of the sites were evaluated and scored at 24 hours post-exposure. Based upon the results, the test substance was use at a dose of 100%.

Induction and Challenge:

Three groups of guinea pigs were prepared and exposed to the test material. For the induction phase, the guinea pigs were induced with dermal application of either distilled water, QST 713 WP (neat), or 0.3% DNCB [1-chloro-2,4-dintrobenzene (in ethanol)]. Each animal received three, six-hour occluded application with 7 days between the applications.

Fourteen days after the last induction exposure, the animals were challenged in the same manner on naive sites according to the following table:

Group	Induction	Challenge		Number of Animals	
		Left Flank	Right Flank	Male	Female
Vehicle control	Distilled Water	QST 713 WP	Distilled Water	5	5
Test Article	QST 713 WP	QST 713 WP	Distilled Water	10	10
Positive Control	(0.3%) DNCB (in ethanol)	(0.2%) DNCB (in acetone)	-	3	3

Eighteen to 24 hours after the challenge, all of the animals were depilated with Neet® Lotion Hair Remover.

Rechallenge

Approximately one week after challenge, the test group animals were rechallenged with the test article (at 100%) at a previously untreated site.

Chemical and Dermal Observations:

All animals were observed for local (dermal) and systemic effects. For the induction phase, each treated site was examined at 24 and 48 hours after each exposure period. For the challenge and rechallenge phases, the test sites were scored a minimum of two hours after depilation (24-hour score). Scoring was repeated 24 hours later (48-hour score).

Body Weight:

Initial and final body weights were recorded for each animal.

Data Evaluation:

A minimum of two of six positive control animals must show a positive reaction (scores ≥ 1) to the control material (DNCB) to validate the test system. Scores of 1 or greater in the test group also indicate sensitization, provided scores of less than 1 are seen in the vehicle control animals. If three or more animals in the test group show evidence of responsiveness in the absence of significant responsiveness in the vehicle control group, the conclusion is that subsequent exposure to humans may involve risk of sensitization. If one or two animals in the test group exhibit positive responses at primary challenge, a rechallenge is recommended.

Incidence is the number of animals in each group showing responses of 1 or greater at either 24 or 48 hours divided by the total number of animals tested in that group.

Severity is calculated as the sum of the test scores divided by the total number of animals tested in a given group (determined at both 24 and 48 hours). Grades of ± are equal to 0.5 for calculation of severity indices. All average grades are rounded off to the nearest tenth. Means and standard deviations were also calculated by group.

II. RESULTS

Table 1. Summary of positive responses* determined during induction phase:

	Week 1	Week 2	Week 3
QST 713	8/20	7/20	3/20
Water	0	0	0
DNCB	3/6	5/6	6/6

* ± or higher scores at 24 and/or 48 hours.

The positive control group provided the anticipated results. All six of the animals exhibited an irritation score of 2 or 3 (3/6 = 2 & 3/6 = 3) at 24 hours and 2/6, 3/6 & 1/6 of the animals exhibited an irritation score of 1, 2, & 3 respectively at the 48 hour observation.

Table 2. Summary of Challenge Results

GROUP	CHALLENGE	SCORE	#ANIMALS/# TESTED	
			24 Hours	48 Hours
Vehicle Control	Distilled Water	0	10/10	10/10
	QST 713 WP	0	8/10	8/10
		±	0/10	2/10
		1	1/10	0/10
		2	1/10	0/10
		3	0/10	0/10
Test Article	Distilled Water	0	20/20	20/20
	QST 713 WP	0	10/20	6/20
		±	6/20	4/20
		1	2/20	6/20
		2	2/20	4/20
		3	0/20	0/20
Positive Control	0.2% DNCB (in acetone)	0	0/6	0/6
		±	0/6	0/6
		1	0/6	2/6
		2	3/6	3/6
		3	3/6	1/6

Scores:
0 = No Reaction
± = Slight Patchy Erythema
1 = Slight or Confluent or Moderate Patchy Erythema

2 = Moderate Erythema
3 = Severe Erythema with/without Edema
There were no signs of systemic toxicity in any group and all animals gained weight during the study.

Table 3. Incidence and Severity at Challenge

Group	Challenge	Incidence	Severity	Incidence	Severity
		24 Hours		48 Hours	
Vehicle Control	Distilled Water	0/10	0.0	0/10	0.0
	QST 713 WP	2/10	0.3	2/10	0.1
Test Article	Distilled Water	0/20	0.0	0/20	0.0
	QST 713 WP	10/20	0.5	14/20	0.8
Positive Control	0.2% DNCB	6/6	2.5	6/6	1.8

- Based on the results of the challenge phase, the test group animals were rechallenged with the test article at 100% approximately one week later.

Table 4. Summary of Rechallenge Results

Group	**Rechallenge**	**24-Hours***					**48-Hours***				
		0	±	1	2	3	0	±	1	2	3
Test Article	QST 713 WP	40	25	25	10	0	70	20	5	5	0
		Incidence = 12/20 Severity = 0.6					Incidence = 6/20 Severity = 0.3				

*Percentage of Animals with Scores After 24 Hours

III. DISCUSSION

The purpose of this study was to determine if the test material, QST 713 WP, has the potential to elicit a delayed dermal contact hypersensitivity response in guinea pigs. The positive control group induced and challenged with DNCB exhibited the expected responses to provide validation of the test methods. Prior to the experimental initiation, the irritation potential of the test material was determined by a dose range study, and based upon the results of this study, the test article was dosed at 100%. Induction with

the test material, QST 713 WP, did elicit a very mild delayed contact hypersensitivity response in guinea pigs which were challenged and rechallenged with the test material.

CLASSIFICATION: ACCEPTABLE. The product should be labeled as a potential dermal sensitizer.

DATA EVALUATION REPORT

Reviewed by: Carl Etsitty, M.S., Microbiologist

Secondary Reviewer: John L. Kough, Ph.D., Senior Scientist

STUDY TYPE:	Manufacturing Process (OPPTS 885.1200)
MRID NO:	448923-01
TEST MATERIAL:	Serenade™WP
PROJECT NO:	None assigned
SPONSOR:	AgraQuest, Inc., Davis, CA 95616
TESTING FACILITY:	AgraQuest, Inc., Davis, CA 95616
TITLE OF REPORT:	Manufacturing and Analytical Data for Serenade™WP
AUTHOR(S):	Laura Cunningham Hilbig, and E.M. Bellet, Ph.D.
STUDY COMPLETED:	July 23, 1999
GOOD LABORATORY PRACTICE:	Non GLP Compliant
CONCLUSION:	[redacted]
CLASSIFICATION:	SUPPLEMENTAL – May be upgraded to ACCEPTABLE, with the following submissions: Proposed range of AI counts; Percent recovery error data to support theoretical calculations, based on lot/batch analysis; Justification of [redacted] limit of detection [redacted]

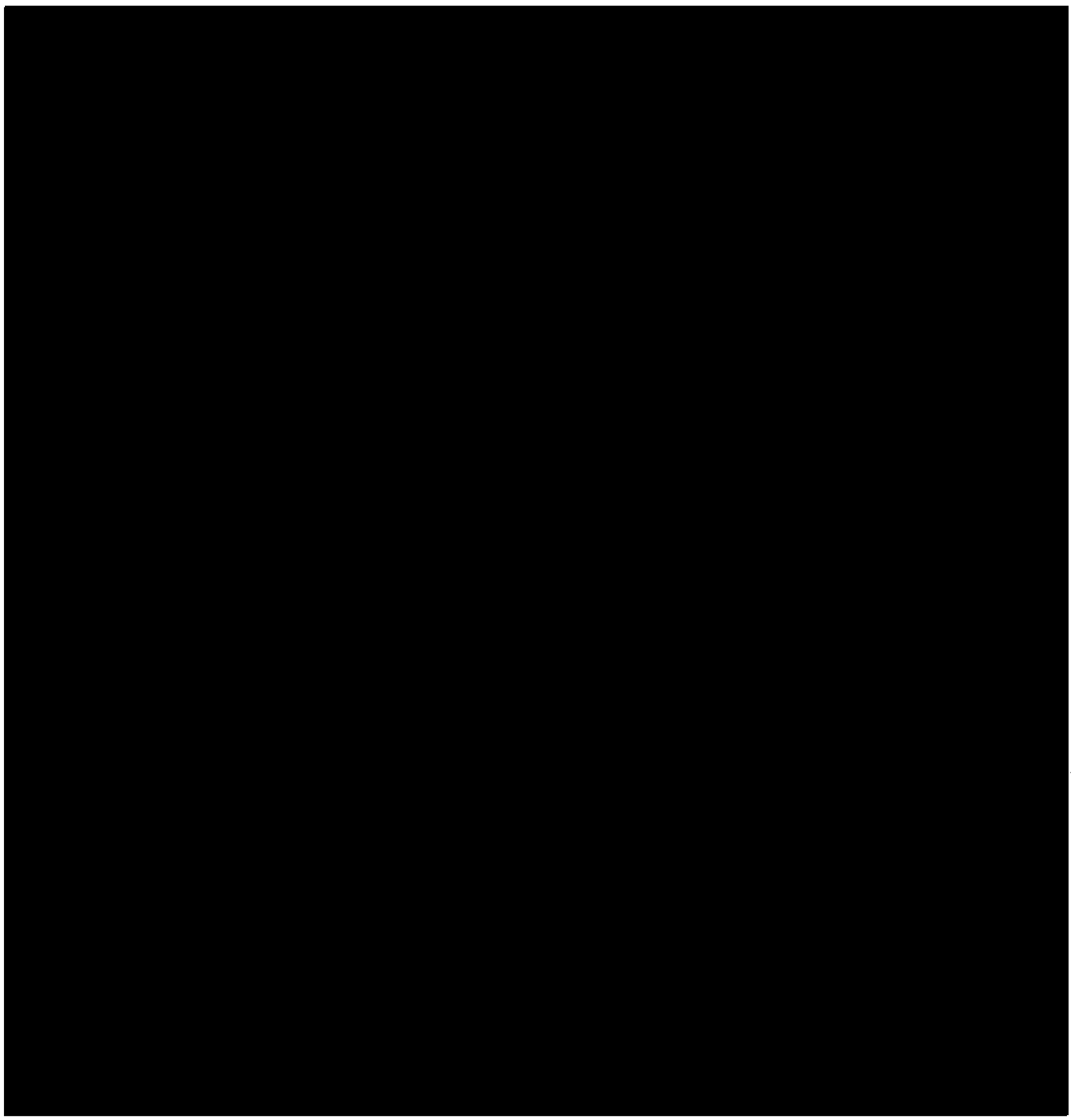
CONTAINS CONFIDENTIAL BUSINESS INFORMATION

Manufacturing process information may be entitled to confidential treatment

1 **STUDY DESIGN**

1.1 Test Material: Serenade™WP, active ingredient is *Bacillus subtilis* Strain QST 713

1.2 **MANUFACTURING PROCESS**:

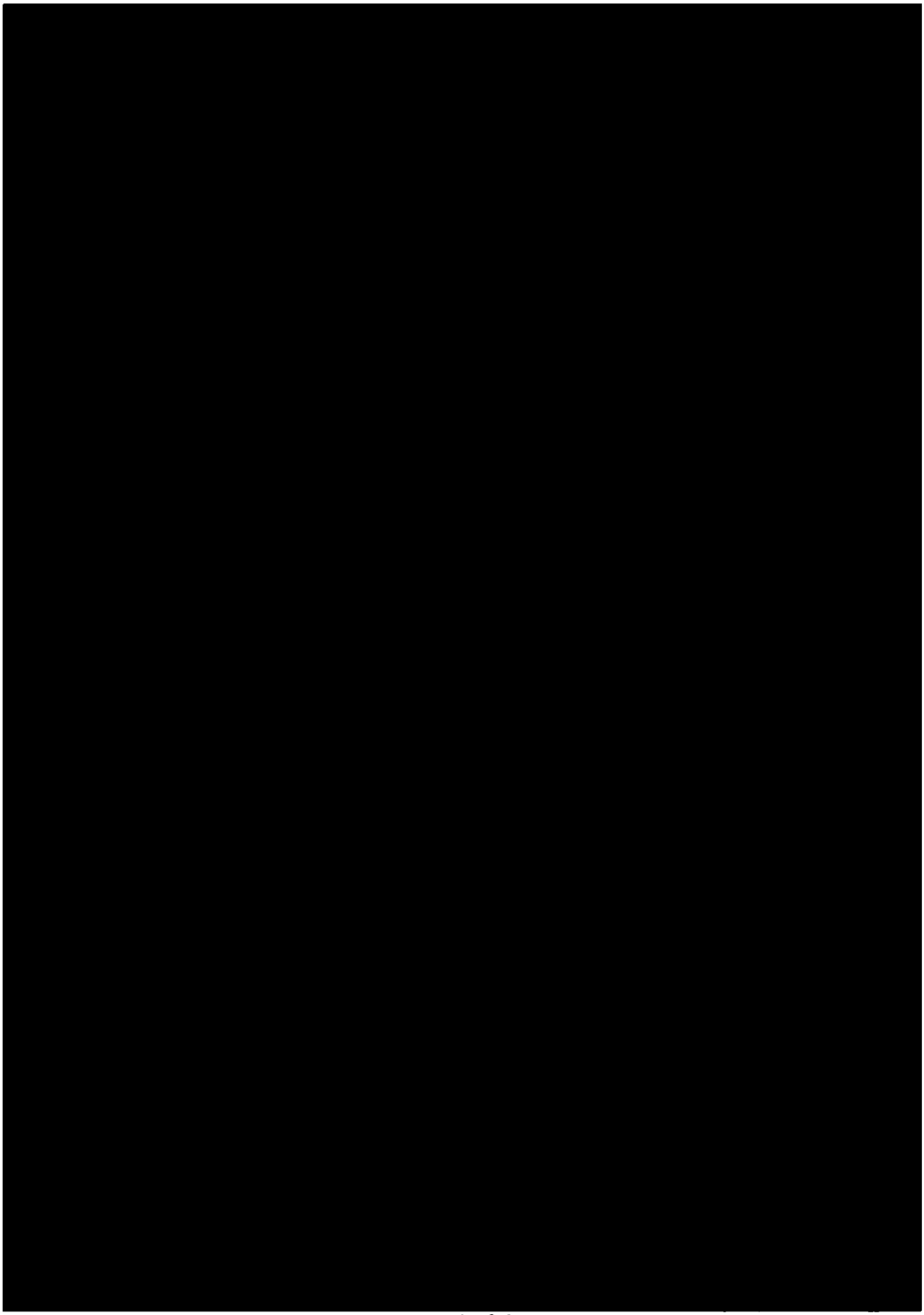


Pages 129-132 - *Manufacturing process information may be entitled to confidential treatment*

[redacted]

8 **DISCUSSION**

[redacted]. The packet is SUPPLEMENTAL, and may be up graded to ACCEPTABLE with the following submissions: Proposed AI cfu range; Percent recovery error data for further justification of theoretical calculations, based on lot/batch analysis; Justification of [redacted] limit of detection [redacted]



Manufacturing process information may be entitled to confidential treatment

DATA EVALUATION REPORT

Reviewed by: Carl Etsitty, M.S., Microbiologist

Secondary Reviewer: John L. Kough, Ph.D., Senior Scientist

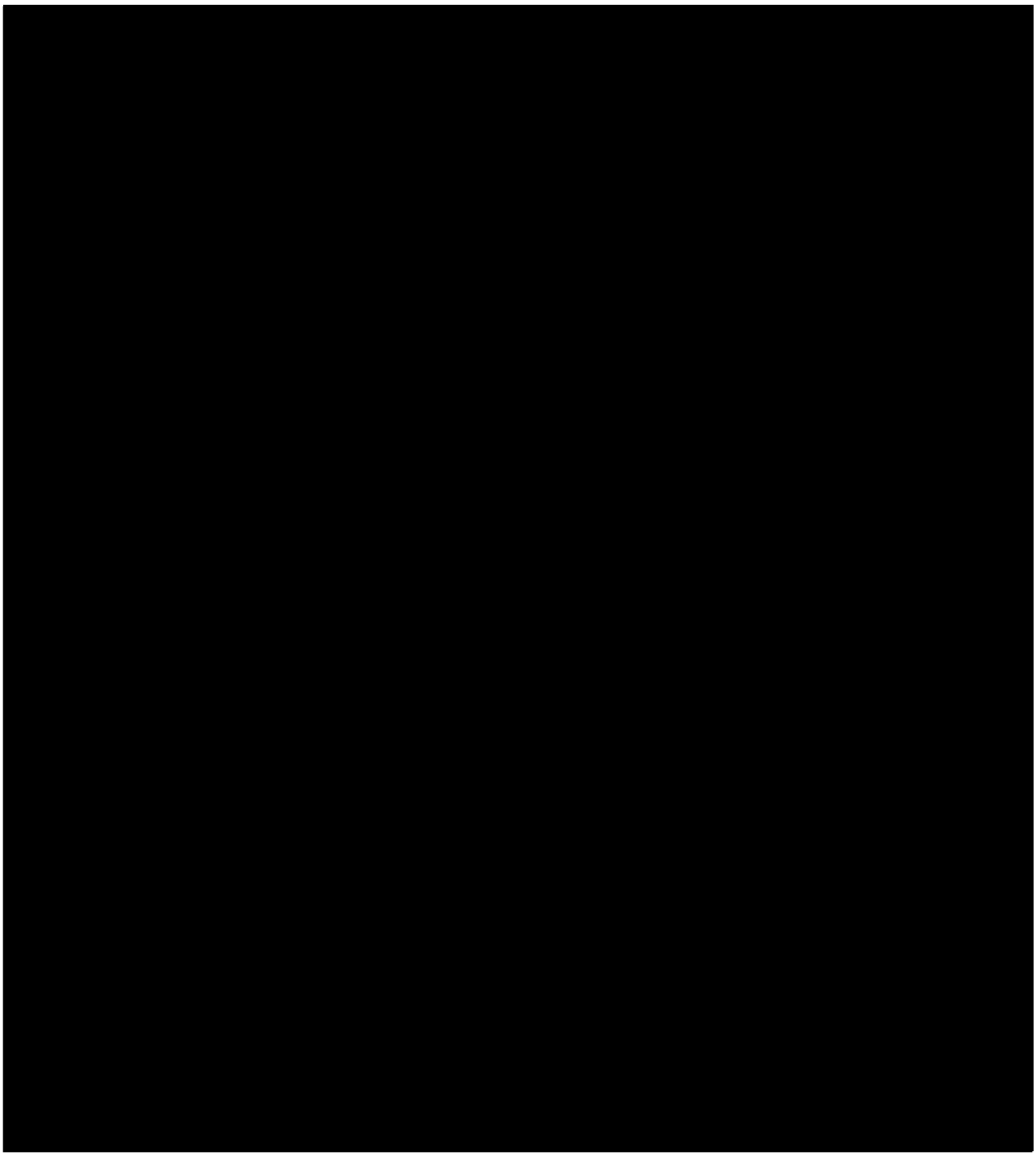
STUDY TYPE:	Manufacturing Process (OPPTS 885.1200)
MRID NO:	448944-01
TEST MATERIAL:	Serenade™WP
PROJECT NO:	None assigned
SPONSOR:	AgraQuest, Inc., Davis, CA 95616
TESTING FACILITY:	AgraQuest, Inc., Davis, CA 95616
TITLE OF REPORT:	Manufacturing and Analytical Data for Serenade™WP
AUTHOR(S):	Laura Cunningham Hilbig, and E.M. Bellet, Ph.D.
STUDY COMPLETED:	July 23, 1999
GOOD LABORATORY PRACTICE:	Non GLP Compliant
CONCLUSION:	[redacted]
CLASSIFICATION:	SUPPLEMENTAL – May be upgraded to ACCEPTABLE, with the following submissions: Proposed AI nominal concentration, with upper and lower limits; Percent recovery error data to support theoretical calculations, based on lot/batch analysis; Justification of [redacted] limit of detection [redacted]

CONTAINS CONFIDENTIAL BUSINESS INFORMATION

1 **STUDY DESIGN**

1.1 Test Material: Serenade™WP, active ingredient is *Bacillus subtilis* Strain QST 713

1.2 **MANUFACTURING PROCESS:**



Manufacturing process information may be entitled to confidential treatment

Pages 137-140 - *Manufacturing process information may be entitled to confidential treatment*

[redacted]

8 **DISCUSSION**

[redacted]. The packet is SUPPLEMENTAL, and may be up graded to ACCEPTABLE with the following submissions: Proposed AI cfu range; Percent recovery error data for further justification of theoretical calculations, based on lot/batch analysis; Justification of [redacted] limit of detection ([redacted]

Manufacturing process information may be entitled to confidential treatment

Lodi Farming Inc.

August 30, 1999

Dr. Janet Andersen, Director
Biopesticides and Pollution Prevention Division (751C)
U.S. Environmental Protection Agency
Office of Pesticide Programs
401 M Street, S.W.
Washington, D.C. 20460

RE: Serenade™ WP Biofungicide

Dear Dr. Andersen:

I am writing you as the President of Mid Valley Apple Association of California.

The Mid Valley Apple Assn. represents 125 growers with over 15,000 acres of apples grown in California's Central Valley. I am also owner and operator of Lodi Farming, Inc. located in Stockton, California.

I am writing this letter to urge you and your staff to complete a timely review of the Serenade Biofungicide registration package. Mid Valley Apple Assn. has followed the progress of the development of Serenade for apples and believes it to be a viable alternative for the control of fire blight, powdery mildew and scab. Registration of this product will provide apple growers with a valuable new tool for crop protection. Implementation of the FQPA with its requirements for reduced risk crop protection products will directly affect our ability to manage our crop on a daily basis. Choices of products available for disease control on apples are limited as registrants amend their labels to balance market demands with regulatory limitations.

The advantages of making AgraQuest, Inc., Serenade Biofungicide available to apple growers are clear. Serenade is effective against such problems as fire blight, powdery mildew and scab and can be applied with conventional application equipment, is compatible with other crop production products and provides another alternative for resistance management programs. In addition, Serenade is an environmentally friendly pesticide whose active ingredient is an ubiquitous microorganism occurring globally. This is the kind of pesticide the U.S. EPA should be expediting through the review process.

If you have any questions and would like to contact me, I can be reached at the address and number below. I would be happy to discuss Serenade's value to the Mid Valley Apple Assn.

Sincerely yours,

Jeff J. Colombini
President, Mid Valley Apple Assn.

CC:
Paul Helliker, Director
Dept. of Pesticide Regulation
830 K Street Mall, Sacramento, CA 95814-3510

11292 NORTH ALPINE ROAD ▪ STOCKTON, CALIFORNIA 95212
TELEPHONE (209) 948-4022 or 334-3424 ▪ FAX: (209) 334-3468

Susanne Correlli
7511C

This document will publish in the FEDERAL REGISTER of 4/26/99.
Approximately 24 hours after publication, page numbers are available by calling the Federal Register Staff (FRS) at 202-260-2253. Please refer to the FRL number at the top of the first page or the number at the bottom left corner of the first page.

Copies of the actual FEDERAL REGISTER publication are made available through the FRS weekly distribution. Extra copies may be made by the interested party from a copy of the FEDERAL REGISTER available in the FRS Office. (NE Mall G-304).

John A. Richards, Director
Federal Register Staff

ENVIRONMENTAL PROTECTION AGENCY

[PF–871; FRL–6074–8]

Notice of Filing of Pesticide Petition

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: This notice announces the initial filing of a pesticide petition proposing the establishment of regulations for residues of a certain pesticide chemical in or on various food commodities.

DATES: Comments, identified by the docket control number PF–871, must be received on or before (*insert date 30 days after date of publication in the* **Federal Register**).

ADDRESSES: By mail submit written comments to: Information and Records Integrity Branch, Public Information and Services Divison (7502C), Office of Pesticides Programs, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. In person bring comments to: Rm. 119, CM #2, 1921 Jefferson Davis Highway, Arlington, VA.

Comments and data may also be submitted electronically by following the instructions under ''SUPPLEMENTARY INFORMATION.'' No confidential business information should be submitted through e-mail.

Information submitted as a comment concerning this document may be claimed confidential by marking any part or all of that information as ''Confidential Business Information'' (CBI). CBI should not be submitted through e-mail. Information marked as CBI will not be disclosed except in accordance with procedures set forth in 40 CFR part 2. A copy of the comment that does not contain CBI must be submitted for inclusion in the public record. Information not marked confidential may be disclosed publicly by EPA without prior notice. All written comments will be available for public inspection in Rm. 119 at the address given above, from 8:30 a.m. to 4 p.m., Monday through Friday, excluding legal holidays.

FOR FURTHER INFORMATION CONTACT: Susanne Cerrelli, Biopesticides and Pollution Prevention Division (7511C), Office of Pesticide Programs, Environmental Protection Agency, 401 M St., SW., Washington, DC 20460. Office location, telephone number, and e-mail address: Rm. 912, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA 22202; (703) 308–8077; e-mail: cerrelli.susanne@epamail.epa.gov.

SUPPLEMENTARY INFORMATION: EPA has received a pesticide petition as follows proposing the establishment and/or amendment of regulations for residues of certain pesticide chemical in or on various food commodities under section 408 of the Federal Food, Drug, and Cosmetic Act (FFDCA), 21 U.S.C. 346a. EPA has determined that this petition contains data or information regarding the

99P-0598

elements set forth in section 408(d)(2); however, EPA has not fully evaluated the sufficiency of the submitted data at this time or whether the data supports granting of the petition. Additional data may be needed before EPA rules on the petition.

The official record for this notice of filing, as well as the public version, has been established for this notice of filing under docket control number [PF–871] (including comments and data submitted electronically as described below). A public version of this record, including printed, paper versions of electronic comments, which does not include any information claimed as CBI, is available for inspection from 8:30 a.m. to 4 p.m., Monday through Friday, excluding legal holidays. The official record is located at the address in ''ADDRESSES'' at the beginning of this document.

Electronic comments can be sent directly to EPA at:

opp-docket@epamail.epa.gov

Electronic comments must be submitted as an ASCII file avoiding the use of special characters and any form of encryption. Comment and data will also be accepted on disks in Wordperfect 5.1/6.1 file format or ASCII file format. All comments and data in electronic form must be identified by the docket control number (PF–871) and appropriate petition number. Electronic comments on this notice may be filed online at many Federal Depository Libraries.

List of Subjects

Environmental protection, Agricultural commodities, Food additives, Feed additives, Pesticides and pests, Reporting and recordkeeping requirements.

Dated: 4-12-99

Janet L. Andersen,

Director, Biopesticide and Pollution Prevention Division, Office of Pesticide Programs.

Summary of Petition

The petitioner summary of the pesticide petition is printed below as required by section 408(d)(3) of the FFDCA. The summary of the petition was prepared by the petitioner and represents the views of the petitioner. EPA is publishing the petition summaries verbatim without editing them in any way. The petition summary announces the availability of a description of the analytical methods available to EPA for the detection and measurement of the pesticide chemical residues or an explanation of why no such method is needed.

AgraQuest,Inc.

PP 8F5032

EPA has received a pesticide petition 8F5032 from AgraQuest, Inc., 1105 Kennedy Place, Davis, California 95616, proposing pursuant to section 408(d) of the Federal Food, Drug, and Cosmetic Act (FFDCA), 21 U.S.C. 346a(d), to amend 40 CFR part 180 to establish an exemption from the requirement of a tolerance for the microbial pesticide *Bacillus subtilis* QST 713 strain in or on all raw agricultural commodities (RAC).

Pursuant to section 408(d)(2)(A)(i) of the FFDCA, as amended, AgraQuest, Inc. has submitted the following summary of information, data, and arguments in support of their pesticide petition. This summary was prepared by AgraQuest, Inc. and EPA has not fully evaluated the merits of the pesticide petition. The summary may have been edited by EPA if the terminology used was unclear, the summary contained extraneous material, or the summary unintentionally made the reader conclude that the findings reflected EPA's position and not the position of the petitioner.

A. Product Name and Proposed Use Practices

Serenade™ WP is being submitted for use as a biofungicide on the following crop groupings:

Curcurbits; Grapes; Hops; Leafy Vegetables (except Brassica); Mushrooms; Peanuts; Peppers; Pome Fruits; Potatoes; Stone Fruits; Strawberries; Tomatoes; Tree Nuts (almonds and pistachios)

B. Product Identity/Chemistry

1. *Identity of the pesticide and corresponding residues.* Serenade™ contains the QST 713 strain of dried *Bacillus subtilis* as the active ingredient. QST 713 Technical is used to formulate Serenade™ WP.

2. *Magnitude of residue at the time of harvest and method used to determine the residue.* Since *Bacillus subtilis* is a ubiquitous organism, it is commonly recovered from soil, water and decomposing plant residue. It is found at population levels of 10^{+6} to 10^{+7} per gram of soil (EPA Risk Assessment of Bacillus subtilis, February, 1997).

3. *A statement of why an analytical method for detecting and measuring the levels of the pesticide residue are not needed.* As formulated in Serenade™ WP, *Bacillus subtilis* will be delivered at $1.0 \times 10^{+6}$ per gram of Serenade™

WP. Therefore, analysis for the organism from use of Serenade™ WP would not be specific and is therefore, not necessary.

C. Mammalian Toxicological Profile

1. *Acute toxicity*—i. Serenade™ WP has been evaluated in an Acute Oral study in male and female Sprague-Dawley Crl:CD (SD)BR rats. No treatment related effects in body weight (bwt) or body weight gain was noted. No clinical signs were noted during the study. Necropsy findings were normal for all male and female rats. The results of this study indicated that the estimated acute oral LD_{50} was greater than 5,000 milligram kilogram (mg/kg).

ii. Serenade™ WP was evaluated as a single dermal dose of 2,000 mg/kg in an acute dermal study in male and female New Zealand White rabbits. There was no mortality observed during the study. Erythema, edema, necrosis, fissuring and/or sloughing of the skin at the application site was noted in all animals. All treated animals exhibited increases in bwt. There were no visible lesions noted in any animal at terminal necropsy. The dermal LD_{50} was estimated to be greater than 2,000 mg/kg.

iii. Serenade™ WP was evaluated in a 4-hour, whole body, acute inhalation study in male and female Sprague-Dawley rats. The maximum concentration (MC) which could be aerosolized was 0.63 milligrams per liter (mg/L), which gave a median aerodynamic particle size of less than 0.4 . No mortality was noted during the study. Some of the clinical abnormalities noted in one or more animals were transient incidences of salvation, breathing abnormalities, decreased activity, wobbly gait, apparent hypothermia, hunched posture, decreased defecation, urine stain, decreased food consumption, and dark material around the facial area. Bwt loss was noted for three female rats (one during the 0-7 day interval, and two during the 7-14 day interval). However, this was a slight bwt loss and was not considered to be biologically significant. No significant gross findings were observed at necropsy. The acute inhalation LC_{50} was estimated to be greater than 0.63 mg/L.

iv. Administration of Serenade™ WP to the eye of New Zealand white rabbits, in a Primary Eye Irritation study, resulted in irritation of the conjunctivae (redness, chemosis, and/or discharge) in all treated animals within 1-hour post-dose. All scores returned to normal by 72 hours post-dose. Therefore, Serenade™ WP is considered to be a mild irritant.

v. In a Primary Dermal Irritation study using New Zealand White rabbits, Serenade™ WP, after a 4-hour exposure, resulted in very slight edema and/or very slight erythema. No other dermal signs were observed. Therefore, Serenade™ WP is considered to be a very slight irritant after 4-hours of exposure.

vi. Serenade™ WP was evaluated in a standard Hypersensitivity study (Buehler) in Guinea Pigs, using Serenade™ WP as received (without any dilution). There were no signs of systemic toxicity in any dose group, and all animals gained weight during the study. Under the conditions of this study,

Serenade™ WP elicited a delayed mild contact hypersensitivity response in guinea pigs when challenged and rechallenged at 100%.

vii. The active ingredient in Serenade™ WP, *Bacillus subtilis*, QST 713 strain, has been evaluated in several pathogenicity studies (acute oral, intravenous, and intratracheal). In the acute oral pathogenicity study there were no deaths noted during the study and necropsy findings were normal for all rats. There was no evidence of pathogenicity or toxicity related to treatment. In the intravenous study in rats, no deaths occurred during the study. There were no treatment related effects noted. The organism was found to significantly clear the body within 35 days. No evidence of toxicity or pathogenicity related to treatment was noted during the course of the study. In the intratracheal study in rats, there was no evidence of toxicity or pathogenicity related to treatment noted during the course of the study.

D. Aggregate Exposure

1. *Dietary exposure*—i. *Food.* Due to the ubiquitous nature of the organism, the concentrations of the organism that already exists in the environment, and the fact that food is already in contact with the organism, the likelihood of increased risk to humans or animals from the use of Serenade™ WP is low.

ii. *Drinking water.* Similarly, exposure to humans from residues of Serenade™ WP in consuming drinking water would be low. The organism is already present in this medium.

2. *Non-dietary exposure.* Exposure to *Bacillus subtilis* in the manufacturing plant (fermentation facility) will be minimal due to rigorous GMP's and quality controls put in place to minimize contamination, cross contamination, and exposure to the workers, and also due to protective equipment worn by manufacturing plant workers. Therefore, inadvertent releases in the workplace would not be expected to increase the risk, especially since high levels of the organism already exist in this environment.

The EPA Risk Assessment of Bacillus subtilis (February, 1997) concludes that "human health and environmental hazards of *Bacillus subtilis* are low" and "the number of microorganisms released from the fermentation facility is low".

E. Cumulative Exposure

Exposure to *Bacillus subtilis* in the manufacturing plant (fermentation facility) will be minimal due to rigorous GMP's and quality controls put in place to minimize contamination, cross contamination, and exposure to the workers, and also due to protective equipment worn by manufacturing plant workers. Therefore, inadvertent releases in the workplace would not be expected to increase the risk, especially since high levels of the organism already exist in this environment. The EPA Risk Assessment of *Bacillus subtilis* (February, 1997) concludes that "human health and environmental hazards of *Bacillus subtilis* are low" and "the number of microorganisms released from the fermentation facility is low".

F. Safety Determination

1. *United States population. Bacillus subtilis* is not pathogenic and pathogenicity data indicate that the organism clears the body significantly within 35 days. Therefore, there would be no increased risk to humans from the expected use of Serenade™ WP.

Serenade™ WP is produced under strict quality controls. The active ingredient is routinely screened for contaminants, including human pathogens. Fermentation raw materials are sterilized before use to eliminate potential contaminants. Antimicrobial agents are included in the formulation to reduce/ eliminate any potential contaminants.

2. *Infants and children.* Since *Bacillus subtilis* is ubiquitous, not pathogenic, causes no human disease, and is considered to be of low risk by the United States EPA, it is unlikely that any harmful effects on children or infants would be expected.

G. Effects on the Immune and Endocrine Systems

Bacillus subtilis is a naturally occurring, non-pathogenic organism which has fungicidal properties. There is no indication that this organism has ever or will ever produce any adverse effect on the human immune or endocrine system. It can be concluded that based upon the existing toxicology, which indicates minimal effects, that there would be no adverse effects on the immune or endocrine systems from the use of Serenade™.

H. Existing Tolerances

Bacillus subtilis GB03 and MBI600 are exempted from the requirements of a tolerance in or on all agricultural commodities when applied as a seed treatment on seeds used for growing crops in accordance with good agricultural practices.

[FR Doc. 99–?????? Filed ??–??–99; 8:45 am]
BILLING CODE 6560–50–F

MAY 20 1999

Attached You Will Find the Index of Documents Submitted Under Docket OPP# PF-871

Notice of Filing a Pesticide Petition

***When Comments are Received, A Copy of the Comments Will be Enclosed.**

Contact the Docket Staff if There are any Questions: (703) 305-5805

*Comments Enclosed: (yes) ✓ (no)_____

NUM	DATE	LNAME	AFFIL	TITLE	PAGES	DOC TYPE
0001	04/26/99	Carelli	EPA	Notice of Filing of Pesticide Petition	3	A
0002	05/10/99	George	U.S. Hop Industry Plant Protection Comm.	Comments Re:	1	A
*** Total ***					4	

02/OPP#PF-871

whchgw@televar.com on 05/10/99 01:27:39 PM

TP

RECEIVED
MAY 10 1999
PUBLIC DOCKET

To: opp-docket@epamail.epa.gov
cc: Laura Sallmen-Smith/DC/USEPA/US@EPA
Subject: Docket Control Number PF-871 - Comments

TO: Biopesticides and Pollution Prevention Division
Environmental Protection Agency - Office of Pesticide Programs

FROM: Ann E. George
U.S. Hop Industry Plant Protection Committee

RE: Docket Control Number PF-871

The U.S. hop industry would like to formally support pesticide petition 8F5032 from AgraQuest, Inc., to establish an exemption from the requirement for tolerance for the biofungicide Serenade in or on all RACs. We also support the use of this product on hops.

Preliminary laboratory testing of this new product in leaf disk assays shows promising results. Although no field efficacy testing has been conducted to date, we are hopeful that Serenade will provide growers with a useful tool for the control of hop powdery mildew.

Thank you for considering these comments.

Ann E. George, Administrator
U.S. Hop Industry Plant Protection Committee
504 N. Naches Ave., #11
Yakima, WA 98901

- whchgw.vcf

191

CPL-399

ENVIRONMENTAL PROTECTION AGENCY

[PF–871; FRL–6074–8]

Notice of Filing of Pesticide Petition

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

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FOR FURTHER INFORMATION CONTACT: Susanne Cerrelli, Biopesticides and Pollution Prevention Division (~~7508W~~ 7511C), Office of Pesticide Programs,

99P-0598

				CONCURRENCES				
SYMBOL	7104	7104	7511	7511C				
SURNAME	H Greene	D. Richards	Hundley	Cerelli				
DATE	3-31-99	3/31/99	4/9/99	4/16/99				

EPA Form 1320-1 (12-70) OFFICIAL FILE COPY

ENVIRONMENTAL PROTECTION AGENCY

[PF–871; FRL–6074–8]

Notice of Filing of Pesticide Petition

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elements set forth in section 408(d)(2); however, EPA has not fully evaluated the sufficiency of the submitted data at this time or whether the data supports granting of the petition. Additional data may be needed before EPA rules on the petition.

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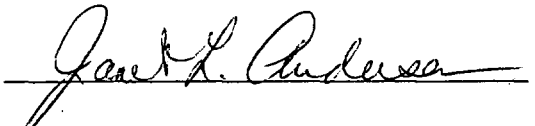
List of Subjects

Environmental protection, Agricultural commodities, Food additives, Feed additives, Pesticides and pests, Reporting and recordkeeping requirements.

Dated: 4-12-99



Director, Biopesticide and Pollution Prevention Division, Office of Pesticide Programs.

Summary of Petition

The petitioner summary of the pesticide petition is printed below as required by section 408(d)(3) of the FFDCA. The summary of the petition was prepared by the petitioner and represents the views of the petitioner. EPA is publishing the petition summaries verbatim without editing them in any way. The petition summary announces the availability of a description of the analytical methods available to EPA for the detection and measurement of the pesticide chemical residues or an explanation of why no such method is needed.

AgraQuest,Inc.

PP 8F5032

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✓ use italics for Bacillus subtilus

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Serenade™ WP is being submitted for use as a biofungicide on the following crop groupings:

Curcurbits; Grapes; Hops; Leafy Vegetables (except Brassica); Mushrooms; Peanuts; Peppers; Pome Fruits; Potatoes; Stone Fruits; Strawberries; Tomatoes; Tree Nuts (almonds and pistachios)

B. Product Identity/Chemistry

1. *Identity of the pesticide and corresponding residues.* Serenade™ contains the QST 713 strain of dried Bacillus subtilis as the active ingredient. QST 713 Technical is used to formulate Serenade™ WP.

✓ Italics

2. *Magnitude of residue at the time of harvest and method used to determine the residue.* Since Bacillus subtilis is a ubiquitous organism, it is commonly recovered from soil, water and decomposing plant residue. It is found at population levels of 10^{+6} to 10^{+7} per gram of soil (EPA Risk Assessment of Bacillus subtilis, February, 1997).

✓ Italics

3. *A statement of why an analytical method for detecting and measuring the levels of the pesticide residue are not needed.* As formulated in Serenade™ WP, Bacillus subtilis will be delivered at 1.0 x 10^{+6} per gram of Serenade™

✓ Italics

WP. Therefore, analysis for the organism from use of Serenade™ WP would not be specific and is therefore, not necessary.

C. Mammalian Toxicological Profile

1. *Acute toxicity*—i. Serenade™ WP has been evaluated in an Acute Oral study in male and female Sprague-Dawley Crl:CD (SD)BR rats. No treatment related effects in body weight (bwt) or body weight gain was noted. No clinical signs were noted during the study. Necropsy findings were normal for all male and female rats. The results of this study indicated that the estimated acute oral LD_{50} was greater than 5,000 milligram kilogram (mg/kg).

ii. Serenade™ WP was evaluated as a single dermal dose of 2,000 mg/kg in an acute dermal study in male and female New Zealand White rabbits. There was no mortality observed during the study. Erythema, edema, necrosis, fissuring and/or sloughing of the skin at the application site was noted in all animals. All treated animals exhibited increases in bwt. There were no visible lesions noted in any animal at terminal necropsy. The dermal LD_{50} was estimated to be greater than 2,000 mg/kg.

iii. Serenade™ WP was evaluated in a 4-hour, whole body, acute inhalation study in male and female Sprague-Dawley rats. The maximum concentration (MC) which could be aerosolized was 0.63 milligrams per liter (mg/L), which gave a median aerodynamic particle size of less than 0.4 . No mortality was noted during the study. Some of the clinical abnormalities noted in one or more animals were transient incidences of salvation, breathing abnormalities, decreased activity, wobbly gait, apparent hypothermia, hunched posture, decreased defecation, urine stain, decreased food consumption, and dark material around the facial area. Bwt loss was noted for three female rats (one during the 0-7 day interval, and two during the 7-14 day interval). However, this was a slight bwt loss and was not considered to be biologically significant. No significant gross findings were observed at necropsy. The acute inhalation LC_{50} was estimated to be greater than 0.63 mg/L.

iv. Administration of Serenade™ WP to the eye of New Zealand white rabbits, in a Primary Eye Irritation study, resulted in irritation of the conjunctivae (redness, chemosis, and/or discharge) in all treated animals within 1-hour post-dose. All scores returned to normal by 72 hours post-dose. Therefore, Serenade™ WP is considered to be a mild irritant.

v. In a Primary Dermal Irritation study using New Zealand White rabbits, Serenade™ WP, after a 4-hour exposure, resulted in very slight edema and/or very slight erythema. No other dermal signs were observed. Therefore, Serenade™ WP is considered to be a very slight irritant after 4-hours of exposure.

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Serenade™ WP elicited a delayed mild contact hypersensitivity response in guinea pigs when challenged and rechallenged at 100%.

vii. The active ingredient in Serenade™ WP, Bacillus subtilis, QST 713 strain, has been evaluated in several pathogenicity studies (acute oral, intravenous, and intratracheal). In the acute oral pathogenicity study there were no deaths noted during the study and necropsy findings were normal for all rats. There was no evidence of pathogenicity or toxicity related to treatment. In the intravenous study in rats, no deaths occurred during the study. There were no treatment related effects noted. The organism was found to significantly clear the body within 35 days. No evidence of toxicity or pathogenicity related to treatment was noted during the course of the study. In the intratracheal study in rats, there was no evidence of toxicity or pathogenicity related to treatment noted during the course of the study.

D. Aggregate Exposure

1. *Dietary exposure*—i. *Food.* Due to the ubiquitous nature of the organism, the concentrations of the organism that already exists in the environment, and the fact that food is already in contact with the organism, the likelihood of increased risk to humans or animals from the use of Serenade™ WP is low.

ii. *Drinking water.* Similarly, exposure to humans from residues of Serenade™ WP in consuming drinking water would be low. The organism is already present in this medium.

2. *Non-dietary exposure.* Exposure to Bacillus subtilis in the manufacturing plant (fermentation facility) will be minimal due to rigorous GMP's and quality controls put in place to minimize contamination, cross contamination, and exposure to the workers, and also due to protective equipment worn by manufacturing plant workers. Therefore, inadvertent releases in the workplace would not be expected to increase the risk, especially since high levels of the organism already exist in this environment.

The EPA Risk Assessment of Bacillus subtilis (February, 1997) concludes that "human health and environmental hazards of Bacillus subtilis are low" and "the number of microorganisms released from the fermentation facility is low".

E. Cumulative Exposure

Exposure to Bacillus subtilis in the manufacturing plant (fermentation facility) will be minimal due to rigorous GMP's and quality controls put in place to minimize contamination, cross contamination, and exposure to the workers, and also due to protective equipment worn by manufacturing plant workers. Therefore, inadvertent releases in the workplace would not be expected to increase the risk, especially since high levels of the organism already exist in this environment. The EPA Risk Assessment of Bacillus subtilis (February, 1997) concludes that "human health and environmental hazards of Bacillus subtilis are low" and "the number of microorganisms released from the fermentation facility is low".

F. Safety Determination

1. *United States population. Bacillus subtilis* is not pathogenic and pathogenicity data indicate that the organism clears the body significantly within 35 days. Therefore, there would be no increased risk to humans from the expected use of Serenade™ WP.

Serenade™ WP is produced under strict quality controls. The active ingredient is routinely screened for contaminants, including human pathogens. Fermentation raw materials are sterilized before use to eliminate potential contaminants. Antimicrobial agents are included in the formulation to reduce/ eliminate any potential contaminants.

2. *Infants and children.* Since *Bacillus subtilis* is ubiquitous, not pathogenic, causes no human disease, and is considered to be of low risk by the United States EPA, it is unlikely that any harmful effects on children or infants would be expected.

G. Effects on the Immune and Endocrine Systems

Bacillus subtilis is a naturally occurring, non-pathogenic organism which has fungicidal properties. There is no indication that this organism has ever or will ever produce any adverse effect on the human immune or endocrine system. It can be concluded that based upon the existing toxicology, which indicates minimal effects, that there would be no adverse effects on the immune or endocrine systems from the use of Serenade™.

H. Existing Tolerances

Bacillus subtilis GB03 and MBI600 are exempted from the requirements of a tolerance in or on all agricultural commodities when applied as a seed treatment on seeds used for growing crops in accordance with good agricultural practices.

[FR Doc. 99–?????? Filed ??–??–99; 8:45 am]
BILLING CODE 6560–50–F

Biopesticides and Pollution Prevention Division

FAX

DATE: November 19, 1998 **NUMBER OF PAGES:** 3

TO: Name: E. M Bellet, Ph.D.

Address: Agraquest, Inc.
1105 Kennedy Place
Davis, CA

Phone 913-381-7611 (FAX Phone 913-383-1027)

FROM: Susanne Cerrelli
US Environmental Protection Agency.
Office of Pesticide Programs
Biopesticides & Pollution Prevention Division (7511W)
401 M St, S.W., Washington, D.C. 20460
Phone 703-308-8077 (FAX 703-308-7026)
Email: CERRELLI.SUSANNE@ pamail.epa.gov

MESSAGE:

Dr Bellet,

Attached is the template I have in my possesion for Notice of filing . However, a copy of this is on the internet.

The following websites have information regarding producing a notice of filing.

http://intranet.epa.gov/oppthome/intrafrs/frdocsup.htm

is a general site, and under this is BPPD's templates and instructions.

BPPD templates are at the below site.

http://intranet.epa.gov/oppthome/intrafrs/doctempl.htm#biopesticides

A pdf version of the template for BPPD's Notice of filing is at the below site
http://intranet.epa.gov/oppthome/intrafrs/biopetit.pdf

Please let me know if you have any trouble accessing these sites. My phone number is 703-308-8077

Susanne Cerrelli

COMPANY FEDERAL REGISTER DOCUMENT SUBMISSION TEMPLATE

[INSTRUCTIONS: Please utilize this outline in preparing tolerance petition submissions. In cases where the outline element does not apply please insert "NA-Remove" and maintain the outline. The comment notes that appear on the left margin represent hidden typesetting codes designed to expedite the processing of the FEDERAL REGISTER document. Please do not remove or alter these codes or change the margins in your submission. Simply type the information requested starting after the heading.

1. [Insert Company Name]

*PP **[insert petition number]***

EPA has received a pesticide petition (PP**[insert petition number]**)from [insert company name and address. proposing pursuant to section 408(d) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. section 346a(d), to amend 40 CFR Part 180

Options (pick one)

1. by establishing a tolerance for residues of
2. to establish an exemption from the requirement of a tolerance for

[insert chemical name] in or on the raw agricultural commodity . The proposed analytical method involves homogenization, filtration, partition and cleanup with analysis by high performance liquid chromatography using UV detection. EPA has determined that the petition contains data or information regarding the elements set forth in section 408(d)(2) of the FFDCA; however, EPA has not fully evaluated the sufficiency of the submitted data at this time or whether the data supports granting of the petition. Additional data may be needed before EPA rules on the petition.

A. Residue Chemistry

1. *Plant Metabolism.*[**insert text**]

2. *Analytical Method.*[**insert text**]

3. *Magnitude of residues.*[**insert text**]

B. Toxicological Profile

1. *Acute toxicity.* [**insert text**]

2. *Genotoxicty.* [**insert text**]

3. *Reproductive and Developmental Toxicity.* **[insert text]**

4. *Subchronic Toxicity.* **[insert text]**

5. *Chronic Toxicity.* **[insert text]**

6. *Animal Metabolism.* **[insert text]**

7. *Metabolite Toxicology.* **[insert text]**

C. Aggregate Exposure

1. *Dietary Exposure.* **[insert text]**

2. *Food.* **[insert text]**

3. *Drinking water.* **[insert text]**

4. *Non-Dietary Exposure.* **[insert text]**

D. Cumulative Effects

E. Safety Determination

1. *US population.* **[insert text]**

2. *Infants and children.* **[insert text]**

F. International Tolerances

FRONT END PROCESSING FORM TO FILE ROOM

DATE 9/4/98

FILE NUMBER 8F5032

_____ No Data - File Room "Make Ready for _____"
(PM or Individual)

✓ Data - File Room "Assign Jacket to Shelf"

_____ Rejected - File Room "Assign Jacket to Rejected Shelf"

AgraQuest, Inc.

1105 Kennedy Place
Davis, CA 95616
tel. 530.750.0150
fax. 530.750.0153
AgraQuest@aol.com

Innovative natural product solutions for pest management

8-240AG

August 28, 1998

Document Processing Desk
ATTENTION: Dr. Janet Andersen
Director
Biopesticides and Pollution Prevention Division
U.S. Environmental Protection Agency
7501W
401 M Street, S.W.
Washington, D.C. 20460

RE: Exemption from Tolerance Petition For Serenade™ WP Biofungicide, USEPA Registration Number 69592-XX.

Dear Dr. Andersen:

The undersigned, AgraQuest, Inc., 1105 Kennedy Place, Davis, California, submits this petition pursuant to Section 408(d) (1) of the Federal Food, Drug and Cosmetic Act for an Exemption from the Requirements of Tolerance for all agricultural uses with respect to the product SERENADE™ WP, EPA Registration Number 69592-XX. The active ingredient in the product is the QST 713 strain of dried *Bacillus subtilis*. The product is formulated to provide a minimum of $1 \times 10^{+6}$ cfu/g.

Included with this transmittal letter you will find the Summary of Information, Data and Arguments in support of this petition. Attached hereto in triplicate and consisting as part of this petition are the following:

A. The name, chemical identity, and composition of the active ingredient in SERENADE™ WP, the manufacturing process, discussion of formation of unintentional ingredients, certification limits, analytical methods and physical and chemical properties of this pesticide product.

B. Amount, frequency and time of application, including draft labelling.

C. Safety studies.

D. Results of tests on Residues.

E. Practical Methods for Removing Residues.

F. Proposed Tolerance.

G. Reasonable Grounds in Support of the Petition.

A submission is also being made, separately, to the Administrator which provides the required fee of $12,100 (Twelve thousand one hundred dollars) associated with this request for an Exemption. A copy of the check submitted in support of this submission is attached.

The product is a biological fungicide containing *Bacillus subtilis* and as such does not present a danger to humans or the environment. Support for this conclusion is based upon statements cited in the Agency's document titled "Final Risk Assessment of *Bacillus subtilis*, February 1997". (The full Assessment is provided under Section G of this petition). *Bacillus subtilis* is "considered a benign organism as it does not possess traits that cause disease." Use of Serenade™ WP, at recommended label rates, would not increase "levels of bacilli normally present (10^{+6} to 10^{+7} per gram) in soils".

The active ingredient in QST 713 Technical is the QST strain of *Bacillus subtilis*. Toxicological studies included with this submission define the active ingredient as QST 713 strain of *Bacillus subtilis* with residual fermentation media. In the preparation of this submission it became apparent that this would cause undo confusion. The residual fermentation media is highly soluble and its level can vary from one production run to another. Therefore, we have excluded the residual fermentation media from the active ingredient and now include it as an inert material. The decision to change our definition of active ingredient to exclude the residual fermentation media was only made after consultation with the EPA and now allows an acceptable range for the active ingredient in QST 713 Technical. It is important to note that this change in description of the active ingredient does not, in any way, affect the composition of the technical product that was used in all of the toxicological studies.

AgraQuest, Inc., is a startup company which specializes in biological products having no environmental concern. We are focusing on fruits, nuts and vegetables, including many minor crops for which there are fewer and fewer products for crop protection. We urgently request an EXPEDITED review of this submission so that we can provide our product to growers for the upcoming season.

If you have any questions or require anything further, please do not hesitate to contact Dr. E. M. Bellet at 913/381-7811.

Thank you for your prompt attention to this submission.

Sincerely,

Pam Marrone, PhD.
President

Jennifer Ryder Fox, PhD.
Director
Regulatory Affairs

E. M. Bellet, PhD.
Consultant

AgraQuest, Inc.

1105 Kennedy Place
Davis, CA 95616
tel. 530.750.0150
fax. 530.750.0153
AgraQuest@aol.com

Innovative natural product solutions for pest management

8-238DG

August 26, 1998

U. S. Environmental Protection Agency
Headquarters Accounting Operations Branch
Office of Pesticide Programs
(Tolerance Fees)
P.O. Box 360277M
Pittsburg, Pennsylvania 15251

RE: Fees For Exemption from Tolerance Petition For Serenade™ WP Biofungicide, USEPA Registration Number 69592-XX.

Dear Sir or Madam:

The undersigned, AgraQuest, Inc., 1105 Kennedy Place, Davis, California, has submitted a petition pursuant to Section 408(d) (1) of the Federal Food, Drug and Cosmetic Act for an Exemption from the Requirements of Tolerance for all agricultural uses with respect to the product SERENADE™ WP, EPA Registration Number 69592-XX.

This submission was sent directly to the U.S. EPA in Washington, D.C. on August 28, 1998.

Enclosed with this letter you will find a check for the amount of $12,100 (Twelve thousand one hundred dollars), AgraQuest check number 5392, dated August 26, 1998.

Thank you for your assistance.

Sincerely,

Pam Marrone, PhD.

Jennifer Ryder Fox, PhD.
Director
Regulatory Affairs

E. M. Bellet, PhD
Consultant

AGRA QUEST, INC.

5392

REFERENCE NO.	DESCRIPTION	INVOICE DATE	INVOICE AMOUNT	DISCOUNT TAKEN	AMOUNT PAID
713		8/26/98	12,100.00		12,100.00
Tolerance					

CHECK DATE	CHECK NO.	PAYEE	DISCOUNTS TAKEN	CHECK AMOUNT
8/26/98	5392	U. S. E. P. A.		$12,100.00

UNION BANK OF CALIFORNIA
11-49/1210

5392

AgraQuest, Inc.
1105 Kennedy Place, Suite 4
Davis, CA 95616
916-750-0150

5392 Aug 26, 1998 *****$12,100.00
CHECK NO. DATE AMOUNT

Twelve Thousand One Hundred and 0/100 Dollars

PAY TO THE ORDER OF: U. S. E. P. A.

AUTHORIZED SIGNATURE

SECURITY FEATURES INCLUDED. DETAILS ON BACK.

⑈005392⑈ [redacted]

B. The amount, frequency, and time of application of the pesticide chemical.

Label for SERENADE™ WP is attached. The active ingredient is present at a minimum of 1.0%.

SERENADE™ WP

Biofungicide

ACTIVE INGREDIENT

QST 713 strain of dried *Bacillus subtilis* . . . . .1% (min)

Inert Ingredients. . . . . .99% (max)

Total 100%

Contains a minimum of 1 x 10^8 cfu/g

EPA Reg. No. 69592-XX

EPA Est. No. 069592-CA-001

Patent pending on QST 713 strain of *Bacillus subtilis* with residual fermentation media.

KEEP OUT OF REACH OF CHILDREN

CAUTION

STATEMENT OF PRACTICAL TREATMENT

IF IN EYES: Flush eyes with a large amount of water. Call a physician if irritation persists.

IF INHALED: Move victim to fresh air.

IF ON SKIN: Wash with plenty of soap and water. Call a physician if irritation persists.

IF SWALLOWED: Drink one or two glasses of water and induce vomiting by touching back of the throat with finger. If person is unconscious, do not give anything by mouth and do not induce vomiting. Call a physician or Poison Control Center.

PRECAUTIONARY STATEMENTS

HAZARDS TO HUMANS & DOMESTIC ANIMALS

CAUTION

Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water. Get medical attention if irritation persists.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

Applicators and other handlers must wear:

- Long-sleeved shirt and long pants
- Gloves
- Shoes plus socks

Follow manufacturer's instructions for cleaning and maintaining PPE. If no instructions are available, use detergent and hot water for washables. Keep and wash PPE separately from other laundry.

USER SAFETY RECOMMENDATIONS

Users should:
Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet.

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling. For any requirements specific to your state or tribe, consult the agency responsible for pesticide regulation.

Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application.

ENVIRONMENTAL HAZARDS

For terrestrial uses, do not apply directly to water or to areas where surface water is present, or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of equipment washwaters.

Do not apply when weather conditions favor drift or runoff from treated areas.

AGRICULTURAL USE REQUIREMENTS

Use this product only in accordance with its labeling and with the Worker Protection Standard 40 CFR Part 170. This Standard contains requirements for the protection of agricultural workers on farms, forests, nurseries and greenhouses and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this label about personal protective equipment (PPE), and restricted entry intervals. The requirements in this box only apply to uses of this product that are covered by the Worker Protection Standard.

Do not enter or allow worker entry into treated areas during the restricted entry interval (REI) of **4** hours.

PPE required for early entry to treated areas that is permitted under the Worker Protection Standard and that involves contact with anything that has been treated, such as plants, soil or water is: coveralls, waterproof gloves, shoes plus socks.

STORAGE, DISPOSAL & SPILLS

STORAGE: Store in a dry, area inaccessible to children. Store in original containers only. Keep container closed when not in use.

PESTICIDE DISPOSAL: Wastes resulting from the use of this product may be disposed of on site or at an approved waste disposal facility. Do no contaminate water when disposing of equipment rinsate.

CONTAINER DISPOSAL: Completely empty bag into application equipment. Then dispose of empty bag in a sanitary landfill or by incineration or, if allowed by State and local authorities, by burning. If burned, stay out of smoke.

GENERAL USE INFORMATION

Serenade™ WP is a broad spectrum, preventive product recommended for the control of many important plant diseases. Serenade™ WP may be applied as a foliar spray alone, in alternating spray programs or in tank mixes with other registered crop protection products. Serenade™ WP may be applied with spray equipment commonly used for making ground applications.

INTEGRATED PEST MANAGEMENT (IPM)

Integrate Serenade™ WP into an overall disease and pest management strategy whenever fungicide use is necessary. Follow practices known to reduce disease development. Consult local agricultural authorities for specific IPM strategies developed for your crop(s) and location.

USE RATE DETERMINATION

Carefully read and follow all label directions, use rates and restrictions. Serenade™ WP should be applied prior to or in the early stages of disease development. Use maximum label rates and shortened spray intervals for conditions conducive to threatening or rapid disease development. For proper application, determine the number of acres to be treated, the recommended label use rate and select appropriate gallonage to give good canopy penetration and coverage of plant parts to be protected. Prepare only the amount of spray solution required to treat the measured acreage. Accurate spray equipment calibration is recommended prior to use.

PREHARVEST INTERVAL

Serenade™ WP can be applied up to and including the day of harvest.

APPLICATION INSTRUCTIONS

GROUND: Thorough coverage is essential for optimum disease control. To achieve good coverage use proper spray pressure, gallonage per acre, nozzles, nozzle spacing and ground speed. Consult spray nozzle and accessory catalogues for specific information on proper equipment calibration.

AERIAL: Not registered for use by aerial application.

CHEMIGATION: Not registered for use through irrigation systems.

MIXING INSTRUCTIONS

MIXING: Serenade™ WP is intended for dilution with water for spray application and may be used in spray equipment commonly used for making ground applications. Partially fill the spray tank with clean water and begin agitation. Add the specified amount of Serenade™ WP to the tank. Finish filling the tank to the desired volume to obtain the proper spray concentration. Maintain agitation continuously while spraying. Do not allow spray mixture to stand overnight or for prolonged periods.

COMPATIBILITY: Do not combine Serenade™ WP in spray tank with pesticides, surfactants or fertilizers unless prior experience has shown the combination physically compatible, effective and non-injurious under conditions of use.

CONDITIONS FOR SALE AND WARRANTY

AgraQuest warrants to those persons lawfully acquiring title to this product that at the time of the first sale of this product by Seller that this product conformed to its description and was reasonably fit for the purposes stated on the label when used in accordance with Seller's directions under normal use conditions. Buyers and users of this product assume the risk of any use contrary to such directions. EXCEPT AS PROVIDED ELSEWHERE IN WRITING CONTAINING AN EXPRESS REFERENCE TO THIS WARRANTY AND LIMITATION OF DAMAGES, SELLER MAKES NO OTHER EXPRESS OR IMPLIED WARRANTY OR GUARANTY, INCLUDING ANY OTHER EXPRESS OR IMPLIED WARRANTY OF FITNESS OR OF MERCHANTABILITY AND NO AGENT OF SELLER IS AUTHORIZED TO DO SO. In no event shall Seller's liability for any breach of warranty exceed the purchase price of the material as to which a claim is made.

Buyers and users of this product are responsible for all loss or damage from use or handling of this product which results from conditions beyond the control of Seller, including, but not limited to, incompatibility with other products unless otherwise expressly provided in the Directions for Use of this product, weather conditions, cultural practices, moisture conditions or other environmental conditions outside the ranges that are generally recognized as being conducive to good agricultural and/or horticultural practices.

Serenade is a registered trademark of AgraQuest.

AgraQuest, Inc.
1105 Kennedy Place
Davis, CA 95616

Recommended Application Rates for Selected Crops

(Serenade ™ WP Biofungicide has a 0-Day PreHarvest Interval for all crops contained on this label)

Crops	Disease	Rate lb/acre	Use Recommendations
Cucurbits	Powdery Mildew *Erysiphe* spp. *Sphaerotheca* spp.	8 – 10	Begin applications when environmental conditions and plant stage are conducive to threatening or rapid disease development. Continue sprays at 7-day intervals or as needed.
Grapes	Gray Mold *Botrytis cinerea* Summer Rot	4 – 8	Apply in sufficient water to provide full coverage. Make applications at bloom, before bunch closure, at veraison and up to day of harvest if necessary.
	Downy Mildew* *Plasmopara viticola*	4 – 8	Apply at 10-inch shoot, 1% bloom to post-bloom (berry set and ¼-inch berry size) and before bunch closure (berry touch).
	Powdery Mildew *Uncinula necator*	4 – 8	Apply in sufficient water to provide thorough coverage starting when new shoots are ½- to 1-½- inches long. Repeat when shoots are 3- to 5-inches long, when shoots are 8- to 10-inches long, and then at 7- to 10-day intervals until disease conditions no longer exist.
Hops*	Powdery Mildew	8 – 10	Begin application in April and apply weekly until harvest.
Leafy Vegetables (except Brassica)	Downy Mildew *Bremia lactucae* *Peronospora* spp.	6 – 8	Begin applications when environmental conditions are conducive to threatening or rapid disease development. Continue sprays at 7-day intervals or as needed.
Mushrooms*	*Trichoderma harzianum*	1,000 ppm	Thoroughly mix throughout growing substrate.
Peanuts*	Early Leaf Spot *Cercospora* spp.	4 – 8	Begin applications when environmental conditions are conducive to threatening or rapid disease development. Peanut hay may be fed to livestock.
Peppers*	Gray Mold *Botrytis cinerea*	5 – 10	Begin applications when environmental conditions are conducive to threatening or rapid disease development. Continue sprays at 7-day intervals or as needed.
	Powdery Mildew *Oidiopsis taurica*	6 – 8	
Pome Fruits	Fire Blight *Erwinia amylovora*	4 – 8	Begin applications at bloom and continue while environmental conditions are conducive to threatening or rapid disease development.
	Scab *Venturia* spp.	8	**Prebloom**: Begin applications at green tip or when environmental conditions become favorable for primary scab development. Apply Serenade™ WP alone or in rotation with another registered fungicide on a 7- to 10-day schedule.
		8	**Postbloom**: Use Serenade™ WP in rotation with the recommended rate of a fungicide registered for use on apples for improved fruit scab and summer disease control.
	Powdery Mildew *Podosphaera leucotricha*	8 – 10	Begin application at tight cluster and continue through the second cover spray. Additional sprays beyond second cover may be needed on susceptible varieties or when environmental conditions are conducive to threatening or rapid disease development. Use high label rate if powdery mildew was present in previous years.
Potatoes	Early Blight *Alternaria solani*	3 – 6	Begin applications when plants are 4- to 6-inches high by applying 3 lbs. per acre. As the vines increase in size, apply 3 to 6 lbs. per acre. Repeat applications at 5- to 7-day intervals or as needed. It is recommended that this product be used within an Integrated Pest Management program. Use maximum label rates under conditions conducive to rapid disease development.
	Late Blight *Phytophthora infestans*	3 – 6	

***Not for use in California**

Crops	Disease	Rate lb/acre	Use Recommendations
Stone Fruits	Brown Rot *Monilinia* spp.	4 – 8	For suppression, apply in sufficient water to provide full coverage at pink bud, full bloom and petal fall stages.
Strawberries*	Gray Mold *Botrytis cinerea*	4 – 8	By ground, apply in sufficient water to provide full coverage at pink bud, full bloom and petal fall stages.
Tomatoes	Early Blight *Alternaria solani* Late Blight *Phytophthora infestans*	4 – 8	Begin applications when environmental conditions are conducive to threatening or rapid disease development. Continue applications on 5- to 7-day intervals or as needed. Use maximum label rates under conditions conducive to rapid disease development.
	Gray Mold *Botrytis cinerea* Powdery Mildew *Levillula taurica*	4 – 8	Begin applications when environmental conditions are conducive to threatening or rapid disease development. Continue applications on 5- to 7-day intervals or as needed.
	Bacterial Spot *Xanthomonas* spp.	4 – 8	For suppression begin applications when environmental conditions are conducive to threatening or rapid disease development. Continue applications on 5- to 7-day intervals or as needed.
Tree Nut Crops: Almonds	Brown Rot, Blossom Blight, Twig Blight *Monilinia* spp.	4 – 8	For suppression, apply in sufficient water to provide full coverage at pink bud, full bloom and petal fall stages. Hulls may be fed to livestock.
Pistachios	Leaf Blight *Alternaria* spp.	4 – 8	For suppression, begin applications in June; repeat on a 30-dayinterval or as needed throughout the season.
	Panicle and Shoot Blight *Botryosphaeria dothidea*	4 – 8	For suppression, begin applications in April; repeat on a 30-day interval or as needed throughout the season.

*** Not for use in California**

F. Proposed Tolerances for the Pesticide Chemical.

We are requesting an exemption from the requirements of a tolerance for SERENADE™ WP for all agricultural uses.

Due to the percentage of active in the formulated product and the biological nature of the product, we do not expect to find any measurable residues, which could be readily attributable to SERENADE™ WP.